Battling Malaria in North-East India Targeting Interventions Towards Elimination

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Dedication

In recognition of services of the fellow mosquito biologists, co-workers and the unknown soldiers who laid their lives in battle against malaria for benefit of future generations living in the malaria-free world.

Contents

Foreword - I

India has made impressive gains in malaria control from six million cases in 1976 (post-resurgence) to 0.18 million reported cases in 2020. The disease distribution and transmission intensities are, however, heterogeneous across the Indian landscape. A larger share of cases is concentrated in the north-east, the east and in the east-central India and these together contribute~80% of the disease burden with predominance of *Plasmodium falciparum*. The north-east region (a group of seven sister states) is considered a corridor for spread of drug-resistant malaria for sharing vast international border and thus have been the focus of attention for its containment from rest of India.

In this context, a network of field units of the National Institute of Malaria Research was established, and these are located in high-risk states. These units have played a pivotal role in understanding the local disease epidemiology and in field-evaluation of newer interventions, viz., long-lasting insecticidal nets (LLINs) for vector control, rapid diagnostic test (RDT) kits, therapeutic efficacy of antimalarial drugs and so on. Based on the evidence-based data generated, these technologies were incorporated in healthcare services resulting in sustainable malaria control and thus making malaria elimination feasible by 2027. The north-eastern state of Assam in particular (with a history of focal outbreaks) has made remarkable gains in transmission reduction over the past decade reporting >90% decline in cases between 2016 to present. The disease transmission trends are steadily in decline in many regions, and this is highly promising for malaria elimination.

This monograph entitled"Battling Malaria in North-East India: Targeting Interventions Towards Elimination" by Dr Vas Dev dwells on epidemiology and control of malaria transmission specific to that region. It encompasses data on technologies that helped to spearhead interventions that drove down transmission to lower levels. It is evident that solutions to malaria lie in continued research efforts and in universalizing interventions. This compilation is a useful resource and guide for young researchers, fellow scientists, and programme managers alike as it will help them formulate policies to address research needs to overcome imminent threats to the elimination initiatives.

Although malaria elimination is feasible and may be within reach, the road to it is not without challenges. This calls for concerted action to erase the asymptomatic reservoirs of

infection, to intensify disease surveillance and to address the funding gap for universalization of interventions. In addition, strengthening of outreach community programmes is vital. With our growing knowledge of disease vectors and transmission dynamics, India today is better equipped to attain its public health goals than ever before. Malaria elimination in India will be a vital and highly significant milestone for the country and our neighbouring regions.

I am very grateful to Dr Vas Dev and his team for their dedication and continued research efforts towards fighting malaria. We stay steadfast in our resolve to conquer malaria.

Amit Sharma, *Ph.D*.
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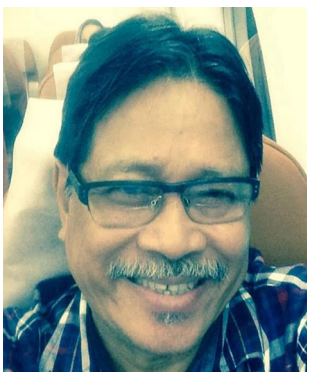
Foreword - II



Historically, North-East India is endemic for malaria and highly receptive for transmission given the conducive climate and host of mosquito species that transmit malaria. Malaria has been the major public health ailment and focus of attention by programme and policy managers incurring huge costs, yet transmission continued unabated. Decades of attempted control interventions including DDT spray for vector containment and antimalarials for treatment of cases failed to disrupt transmission. Post-resurgence 1976, operational costs were seen escalating and returns diminishing. Focal disease outbreaks were returning with vengeance and spreading in areas hitherto unaffected amidst public outcry affecting industrial productivity and equitable socio-economic development of the region. Communities had turned hostile perpetuating vicious cycle of malaria and poverty. Since then, lot more had changed in terms of land use pattern and population explosion at expense of deforestation, but evidence-based data on changing disease epidemiology and control were scarce and obsolete.

It was amply clear that 'business as usual' approach will not suffice to contain upsurge of malaria, instead alternative strategy was deemed necessary to target situation-specific interventions both against the causative malaria parasites and the carriers, the mosquito vectors. In this context, establishment of field unit of the ICMR – National Institute of Malaria Research in Assam proved to be a milestone in history of malaria control in North-East region. Systematic research investigations on vector ecology and transmission dynamics de novo added significantly to the existing knowledge on local disease epidemiology creating an enabling environment to formulate renewed policy for effective control. Number of newer interventional technologies were put to field-evaluation in close coordination with the state health authorities to name a few: (i) insecticide-treated netting materials/long-lasting insecticidal nets for vector control, (ii) monitoring therapeutic efficacy of antimalarials for upgraded drug-policy to check spread of drug-resistant malaria, (iii) rapid diagnostic test kits for improved disease surveillance, (iv) promoting larvivorous fish as an component of integrated disease vector control; upscaling of all these technologies had resulted in substantial disease transmission reduction. In the past decade, state of Assam, which once carried brunt of the disease burden, has made impressive gains reporting deaccelerating transmission trends. Currently, most districts are reporting case incidences <1 per thousand population and approaching near elimination which could prove to be a harbinger of success to end malaria in the country.

This monograph, "Battling Malaria in North-East India: Targeting Interventions Towards Elimination" by Dr Vas Dev, whom I had interacted all throughout my tenure, elaborates eloquently on various aspects of 'Epidemiology and Control of Malaria' specific to North-East India providing additional insights on the subject, is indeed a monumental contribution. It would be a good resource document for scholars, programme and policy managers helping formulate interventions in time and place averting cases helping to move forward with agenda of malaria elimination. It evolved that research is an essential pillar of strength invigorating newer interventions that are community-based, doable and socially acceptable for sustainable control. Communities must be at the centre stage and empowered for unified action to step up efforts in keeping malaria at bay.

With the available body of knowledge on disease vectors and causative parasites, the control programme is well equipped to outdo the emerging challenges in defeating malaria. It is time to deliver and strengthening healthcare services in reaching out the outreach population groups most at risk. Malaria is preventable and curable; no one should die of malaria, each one and everyone should have access to prevention and treatment irrespective of financial and legal status. With this resolve, malaria elimination is possible, feasible, and we can make it happen soon.

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Foreword - III

Malaria has plagued the humanity for centuries. Ever since Noble Prize-winning discovery that malaria is transmitted by mosquitoes by Sir Ronald Ross in 1847 (accomplished in India), volumes of research activities centred around *Anopheles* (the vector) and *Plasmodium* (the causative parasite); but both outwitted the tropical world by evolving resistance. It was at the turn of the 21st century that ushered in new era of hope with the advent of newer intervention tools namely 'Insecticide-Treated Netting Materials' for vector control and 'Artemisinin-based Combination Therapies' for treatment of drug-resistant malaria; both helped mounting a decisive attack to disrupt the chain.

Historically, India had its own share of successes and failures in malaria control and still accounts for bulk of disease burden (88% of reported cases) in South-East Region of the World Health Organization. India is a vast country and malaria transmission is complex governed by multiplicity of disease vectors and varied contextual determinants. Disease transmission is heterogenous with large concentration of cases in the East, East-Central and North-Eastern states contributing nearly 80% of reported cases and deaths.Among these, North-East (NE) zone is highly receptive for malaria co-inhabiting highly anthropophilic vector species, *Anopheles minimus* (perennial species) and *An. baimaii*, a member species of the *An. dirus* complex (monsoon species); both are efficient carriers. NE region is co-endemic for both *Plasmodium falciparum* and *P. vivax*, yet *P. falciparum* (killer parasite) is the predominant infection in Assam, Meghalaya, Mizoram and Tripura sharing international border with Bangladesh.

NE zone is of economic significance and strategically important for sharing vast international border and considered corridor for spread of drug-resistant malaria to peninsular India and beyond. The region is historically endemic replete with history of devastating disease outbreaks and insurmountable morbidity. Decades of attempted control were all in vain for myriad of operational constraints including inhospitable terrain, inclement weather and restricted access, low-literacy levels, and lack of community awareness on disease prevention and control. Information on disease epidemiology and control aspects was scarce leaving many knowledge gaps unaddressed. This monograph, 'Battling Malaria in North-East India: Targeting Interventions Towards Elimination', attempts to fill the void and traverses through the tale of malaria specific to NE India including an account of population demographics, prevalence of malaria, transmission dynamics, *Anopheles*

mosquito fauna, vectors bionomics and disease transmission relationships, landscape epidemiology, climate change and ecological succession, and available modern control options. Each chapter provides in-depth insights on the subject supported by field-based observations/illustrations and host of up-to-date references for benefit of researchers.

The book elaborates on evidence-based newer intervention tools, viz., long-lasting insecticidal nets (LLINs) for vector control, artemisinin-based combination therapies (ACTs) for treatment of drug-resistant malaria, and alternate diagnostics; rollout of which have helped achieve substantial transmission reduction what was considered formerly invincible. It also highlights the role of health education eliciting community participation and human resource development for sustained efforts in keeping malaria at bay and brings out clearly the emerging biological threats and plausible solutions to strengthen healthcare services to step up efforts to defeat malaria.

Given the evidence-based intervention tools, NE has made laudable achievement in reducing malaria transmission substantially aiming elimination in the forseeable future. Among NE states, Assam (accounting for 66% population share of NE region) has given clear lead reporting 90% decline from 5281 cases in 2017 to 484 cases in 2020, what was formerly considered intractable. Eliminating malaria in NE India would prove to be a big step forward lest we miss the opportunity by complacency alone; instead, it is opportune time to invest heavily up-scaling interventions ensuring 'universal coverage' leaving no one devoid of healthcare access.

This monograph is a research-based document specific to NE India solely contributed by Dr. Vas Dev whom I had interacted significantly helping address the core-issue in disease epidemiology, which he had accomplished by his perseverance and sheer commitment in this discipline of research spanning three decades. This is indeed a monumental work and would be useful resource document for programme managers, academicians and medical arthropodologists alike helping invigorate newer intervention tools and formulating policy towards common gaol of 'freedom from malaria'.

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Preface

North-East India is of strategic importance for sharing vast international border (99% of its total geographical limits) endowed with evergreen rainforest, diverse fauna and flora. This region is highly receptive for malaria transmission owing to climatic determinants and prevalence of disease vectors that are highly anthropophilic. Disease outbreaks were recurring taking heavy toll on human lives deterring equitable socio-economic development of the region. Post-resurgence 1976, interventions '*business as usual*' including indoor residual spraying and disease surveillance were not yielding tangible results and transmission continued unabated. Operational costs were seen rising and returns diminishing amidst public chaos and outcry. While disease surveillance was grossly inadequate, entomological surveillance was virtually non-existent least the monitoring therapeutic efficacy of antimalarial drugs letting the drug-resistant foci proliferate. There was clear paucity of information on basic tenants of disease epidemiology in the changing ecological context given the population explosion, migration across borders, deforestation and changing agricultural practices. In this backdrop, malaria was reckoned as the major public health concern specific to North-East India evidenced by persistent transmission and increasing morbidity and attributable mortality.

Taking cognizance of the issues, systematic investigations were undertaken on various aspects of 'Disease Epidemiology and Control' afresh for data based in high-risk areas under the auspices of the National Institute of Malaria Research (formerly Malaria Research Centre), an apex organization of the Indian Council of Medical Research (ICMR). Data were generated on prevalence of malaria, transmission dynamics, drug-resistance in malaria parasite, disease vectors and bionomical characteristics enabling field-evaluation of alternate interventions that are community-based, sustainable and cost-savvy to corner the disease onslaught. Health education eliciting community participation and continuing education and training programmes for the state health personnel remained the core-activity as an integral component of the'Integrated Disease Vector Control (IDVC)'strategy. Research efforts were focussed to address gaps of information in close coordination with the state health authorities ensuring compliance and smooth transfer of newer interventional technologies in healthcare services. The book comprises 18 chapters grouped into seven sections with major theme; each chapter is self-illustrative treatise made easily discernible by the readers. Included in this 'monograph' is an updated account of research activities on malaria epidemiology (inclusive those of sister organizations based in North-East India) and salient findings for benefit of risk-communities, stakeholders, researchers and programme and policy managers as a matter of record creating an enabling environment spearheading intervention to realize the goal of malaria elimination by target date of 2027, three years ahead of global agenda.

Collectively in the past few decades, a lot of information has been generated on disease epidemiology and control helping better targeting interventions and utilization of resources globally [1-3]. The present monograph is largely an eye-witness account and first-hand ground information by the author for working in high-risk areas not only of Assam but also other north-eastern states for the past three decades (1988-2016) as ready reckoner helping prioritize interventions and allocation of resources to defeat malaria. Throughout the manuscript, language easily read by the commoner has been used to underscore the message that '*zero malaria starts with me*' promoting community participation and involvement in the decision-making process. Number of interventional technologies, viz, long-lasting insecticidal nets (LLINs) for vector control, artemisinin-based combination therapies (ACTs) for treatment of drug-resistant malaria, rapid diagnostic tests (RDTs) for on-the-spot diagnosis, were put to field-evaluation; all of which were incorporated in the state healthcare services resulting in substantial disease transmission reduction each passing year. In North-East India, malarial threat is truly receding with many districts reporting <1 parasite incidence per 1000 population, the lowest ever to date [4].

In the present-day means of communication, it is time to put the best foot forward to reach out the outreach communities by strengthening healthcare services in the periphery for reducing morbidity and preventing deaths. No one should die of malaria, each one and everyone should have access to affordable treatment regardless of legal or financial status. It is the opportune time to move away from the dogma of 'one-size-fits-all' rather focus on tailored solutions based on local data and step-up efforts in diagnosing and treating residual cases adhering to strict timelines within specified period. Building stronger health systems towards 'One Health' based on collaborative, multisectoral and transdisciplinary approach working at the local, regional, national, and global levels with the goal of achieving optimal health, has become central in the climate change scenario.

Given the present-day knowledge and available tools, malaria eradication is well within reach but not without challenges. Malaria parasite is continually evolving, and insecticide-resistant vector populations are establishing foothold. Complacency at this stage will costs heavily in losing the battle almost won. Newer tools are warranted to overcome paradigms of extra-domiciliary transmission and treating asymptomatic cases to break the chain of vicious cycle of malaria and poverty. This monograph is intended for new generation of scientists providing available knowledge on disease epidemiology and control in this part of the world helping invent newer intervention tools and prioritizing resources to defeat malaria towards shared vision of living in the 'malaria-free world'.

This volume is an outcome of my interactions with distinguished Professor Sylvie Manguin (Montpellier, France) and late Dr. V. P. Sharma (founder director of the ICMR - National Institute of Malaria Research, New Delhi), and fraternity of scientists and colleagues (my alma mater) who have been constant source of inspiration. I am highly indebted to Dr. Amit Sharma (present Director of the National Institute of Malaria Research, New Delhi), Dr P. L. Joshi, former Director of the National Vector Borne Disease Control Programme, Delhi and Dr. Dhruba Hojai (former Director Health Services, Government of Assam) for contributing 'Foreword' to this compilation. I would like to acknowledge the support of Dr. B. K. Tyagi (Ex-Director, ICMR - Centre for Research in Medical Entomology, Madurai) and Dr. Vijay Veer (Ex-Director, Defence Research Laboratory, Tezpur) providing significant inputs putting together this volume in order, and to Drs Anil Prakash, Prafulla Dutta, S.K. Ghosh, S. K. Sharma, R. M. Bhatt, Sunil Dhiman, D. R. Bhattacharyya, P. K. Bharti,

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Given the research inputs, North-East has made remarkable gains in reducing malarial threat [5], but much more can be achieved with the universal access to modern tools, viz., insecticide-treated nets for malaria vector control and rollout of artemisinin-based combination therapies for treatment of malaria coupled with drive for increased community awareness and participation. It is time to deliver and reach out the outreach communities at stake; together we can realize the target 'Zero Malaria -Draw the Line Against Malaria'.

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Abbreviations and acronyms

ABER	annual blood examination rate
ABITA	Assam Branch Indian Tea Association
ACT	artemisinin based combination therapy
ACR	adequate clinical response
AES	acute encephalitis syndrome
AI	anthropophilic index
AL	artemether + lumefantrine
An.	*Anopheles*
API	annual parasite incidence
APLMA	Asia Political Leaders Malaria Alliance
APMEN	Asia Pacific Malaria Elimination Network
AS	artesunate
AS-AQ	artesunate-amodiaquine
AS-MQ	artesunate-mefloquine
ASHA	accredited social health activist
ASP	artesunate + sulfadoxine-pyrimethamine
ASPCR	allele specific polymerase chain reaction
AS-PY	artesunate-pyronaridine
ATSB	attractive toxic sugar baits
AQ	amodiaquine
BCC	behaviour change Communication
BS	*Bacillus sphaericus*
BT-malaria	benign tertian malaria
Bti	*Bacillus thuringiensis israelensis*
C	carbamate
CDC	Centre for Disease Control & Prevention
CHW	community health worker
CI	confidence interval
CO II	cytochrome oxidase II
CSP	circum-sporozoite-protein
CQ	chloroquine
CS	capsule suspension
D3	D3 domain of 28S rDNA
DAPI	4′, 6-diamidino-2-phenylindole
DDT	dichloro-diphenyl-trichloroethane
DHA-PPQ	dihydroartemisinin-piperaquine
DHFR	dihydrofolate reductase
DHPS	dihydropteroate synthetase
DNA	deoxyribonucleic acid
EDPT	early diagnosis prompt treatment
EIR	entomological inoculation rate
ELISA	enzyme-linked immunosorbent assay
ETF	early treatment failure
E-2020	countries targeting malaria elimination by 2020
FBO	faith based organization
FDC	fixed dose combination
GFATM	Global Fund to fight AIDS, Tuberculosis and Malaria
GIS	Geographical Information System
GMAP	Global Malaria Action Plan for malaria free World
GMS	Greater Mekong Subregion
GPARC	Global Plan for Artemisinin Resistance Containment
GPIRM	Global Plan for Insecticide Resistance Management
GPS	Global Positioning System
GR	geographical reconnaissance
HBHI	high burden to high impact
H&FW	Health & Family Welfare
HRP 2	histidine-rich protein 2
ICMR	Indian Council of Medical Research
IDSP	Integrated Disease Surveillance Project
IDVC	Integrated Disease Vector Control
IEC	information education & communication
IRS	indoor residual spray
ITN	insecticide treated net
ITPS	insecticidal-treated plastic sheeting
ITS 2	internal transcribed spacer 2
IVM	integrated vector management
JE	Japanese Encephalitis
kdr	knockdown resistance gene
LLIN	long-lasting insecticidal net
LTF	late treatment failure
m	metre
MAL	malathion
MBR	mosquito biting rate
MDA	mass drug administration
MEI	Malaria Elimination Initiative
mtDNA	mitochondrial DNA
MT-malaria	malignant tertian malaria

MPI	monthly parasite incidence
msl	mean sea level
MQ	mefloquine
MPO	modified plan of operation
NE	North-East
NEC	north-east council
NEFA	North-East Frontier Agency
NGO	non-government organization
NHM	National Health Mission
NIMR	National Institute of Malaria Research
NVBDCP	National Vector Borne Disease Control Programme
NMCP	National Malaria Control Programme
NMEP	National Malaria Eradication Programme
NN	neonicotinoid
OC	organochlorine
OP	organophosphate
ONGC	Oil & National Gas Commission
P.	*Plasmodium*
PBO	piperonyl butaoxide
PCD	passive case detection
PCR	polymerase chain reaction
PDA	personal digital assistant
Pf	*Plasmodium falciparum*
PfCP	*Plasmodium falciparum* Containment Programme
Pfhrp 2	*Plasmodium falciparum* histidine-rich protein 2
PCR	polymerase chain reaction
PHC	Primary Health Centre
pLDH	*Plasmodium* lactate dehydrogenase
POP	persistent organic pollutants
PPP	public private partnership
PQ	primaquine

PT	Partec test
Pv	*Plasmodium vivax*
QBC	quantitative buffy coat
RACD	reactive case detection
RBC	red blood corpuscles
RBM	Roll Back Malaria
rDNA	ribosomal DNA
RDT	rapid diagnostic test
RFLP	restricted fragment length polymorphism
RMRC	Regional Medical Research Centre
SCAR	sequence characterized amplified region
SDSS	spatial decision support system
SE Asia	South-East Asia
SFR	smear falciparum rate
SIDA	Swedish International Development Authority
SIRM	sterile insect release method
s.l.	sensu lato
SMC	seasonal malaria chemoprevention
SNP	single nucleotide polymorphism
SP	sulfadoxine-pyrimethamine
SPR	smear positivity rate
s.s.	sensu stricto
SVR	smear vivax rate
TAC	Technical Advisory Committee
TE	Tea Estate
UCSF	University of California San Francisco
USAID	United States Agency for International Development
UMS	Urban Malaria Scheme
VCRC	Vector Control Research Centre
WG	water dispersible granules
WHO	World Health Organization
WHOPES	WHO Pesticide Evaluation Scheme
WP	wettable powder

Background Information

Introduction

Malaria has plagued the humanity for centuries taking heavy toll on account of morbidity and attributable mortality globally. Decades of research efforts have helped contain the disease spread but transmission is continuing unabated in many tropical countries [1]. India is no exception with history of devastating outbreaks, control of which culminated in epoch-making discoveries on mosquito vector biology and role they play in disease transmission [2].

Malaria control is an operational issue involving implementation and execution of interventions in place and time. Both parasite and mosquito vectors have evolved with time evading interventions in force resulting in continuing transmission and upsurge of cases. Malaria has been stymying authorities with disappearance and resurgence with ugly face rendering intervention tools ineffective manifested by insecticide resistance in disease vectors and parasite evolving multidrug-resistant [3]. Operational costs are seen rising making control interventions unsustainable with increasing population push and pull in urban areas. Malaria control has become a complex enterprise and diversified in various ecotypes cutting across several paradigms, viz., rural malaria, urban malaria, forest malaria, tribal malaria, border malaria requiring situation-specific intervention strategies.

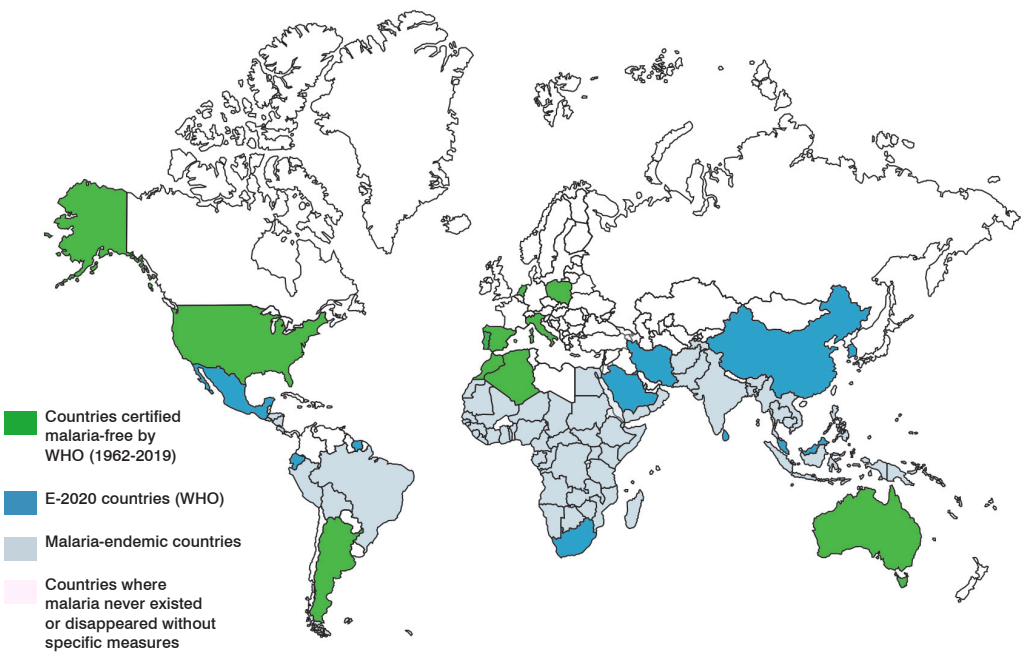

Figure 1. World map showing the World Health Organization certified malaria-free countries between 1962 and 2019 (green) and the E-2020 countries (blue). Source: Sylvie Manguin, Montpellier, France.

With the advent of modern tools and new insights on disease vectors [4], malaria has been cornered targeting elimination by 2030 [5]. Many countries have already been certified malaria-free by the World Health Organization (WHO) and several others reaching malaria elimination soon (Figure 1). India too has made huge strides registering appreciable transmission reduction over the past few years from two million cases in 2016 to less than a half million cases in 2019 and accelerating towards elimination in the foreseeable future [6]. Malarial map in India is shrinking with many states approaching pre-elimination and others reporting >80% decline once considered intractable.

This unprecedented development is based on a solid foundation of evidence-based technologies that were field-evaluated in different geo-epidemiological conditions of the country. Systematic research and evaluation spanning over 30 years of efforts resulted in a host of technologies that were incorporated in healthcare services based on field-demonstration and social acceptance by the communities most at risk. Business as usual encompassing disease surveillance and treatment (anti-parasite measures) and spraying operations (anti-vector interventions) were not yielding quantifiable results and disease transmission was seen never ending affair incurring huge costs [7].

There was an imperative need for alternate interventions that were cost-effective, community-based, and self-sustainable. This task was trusted to the National Institute of Malaria Research (formerly Malaria Research Centre) jointly funded by the Indian Council of Medical Research (an apex organization of the Ministry of Health & Family Welfare for health research) and the Ministry of Science & Technology of Government of India as long-term multicentre project popularly known as 'Integrated Disease Vector Control (IDVC)' project [8]. The mandate was to field-test newer interventions in different ecological transmission settings in close coordination with the respective state health directorate targeting malaria in high-risk regions. It was a mission project using environmentalist approach encompassing an integrated multipronged strategy for malaria control in the Gandhian way of life that is community-based and sustainable (Figure 2).

Figure 2. Components of Integrated Disease Vector Control (IDVC) strategy for malaria vector control. IEC = Information, Education and Communication; ITN = Insecticide Treated Nets; LLIN = Long Lasting Insecticidal Nets. Source Reference [9]

Accordingly, beginning 1986, a network of 'Field Stations' was established in different physiographic zones of the country targeting both urban and rural malaria prioritizing areas reporting high rise in cases and deaths (Figure 3). Amongst varied geo-epidemiological zones, the North-East (NE) region consistently contributed huge burden of cases and was considered invincible for high receptivity and inhospitable terrain. In the seven sister states of NE (Arunachal Pradesh, Nagaland, Assam, Meghalaya, Manipur, Mizoram and Tripura), Assam is the major constituent state with 66% of NE population share and was contributing nearly 50% of the disease burden alone. Taking cognizance of the myriad of issues, number of interventional technologies were subject to field-test in Assam as demonstrative research project later to be transferred to other NE states to launch a decisive attack to defeat malaria for good. Given the mandate, a systematic approach was launched to understand the epidemiology (disease distribution and determinants) afresh based in typically high-risk foothill primary healthcare centre to investigate transmission dynamics to help foot newer interventions both against malaria vectors and parasites to mitigate the disease onslaught.

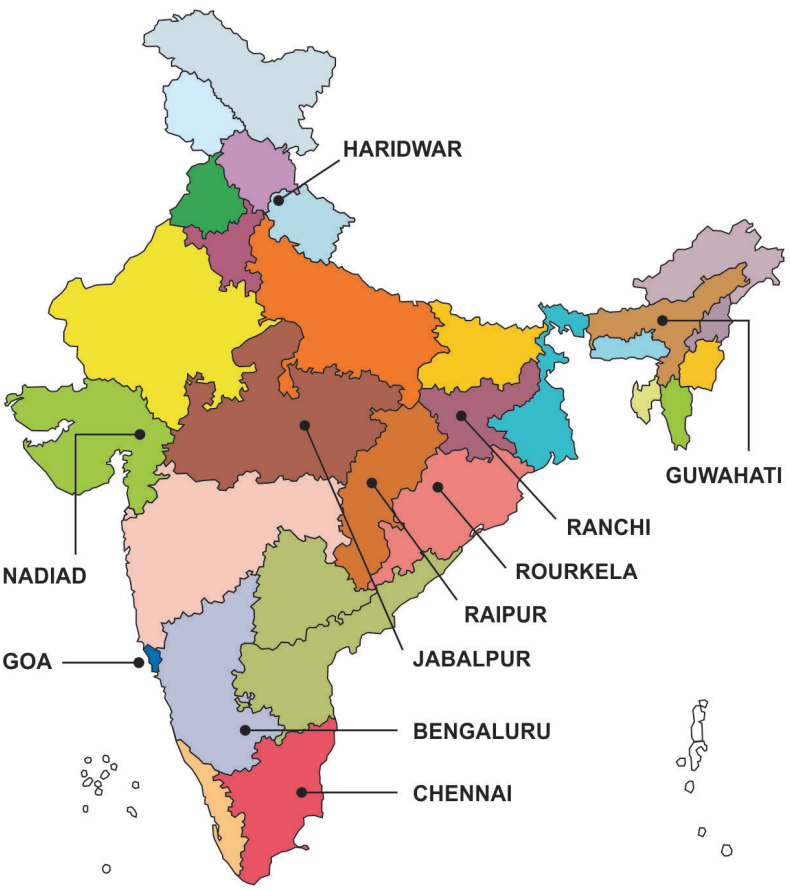

Figure 3. A network of Field Stations of the ICMR - National Institute of Malaria Research (NIMR) located in different physiographic zones of India.

Specific to NE region, the major thrust areas included evaluation of insecticide-treated nets (ITNs) for vector control and monitoring therapeutic efficacy studies of newer antimalarials for treatment of drug-resistant malaria. Other tools included evaluation

of dipsticks (rapid antigen test kits) for early diagnosis enabling prompt treatment, and development of health education modules, viz., booklets, pamphlets, and documentaries that were telecasted during high-transmission seasons to create awareness at the grassroots. In addition, training and re-orientation programmes were held periodically for benefit of the state health functionaries.

ITNs were runaway success in reducing human vector contact disrupting transmission and were widely accepted by the communities at large [10]. Monitoring therapeutic efficacy of antimalarials resulted in periodic upgradation of drug-policy for radical cure of *Plasmodium falciparum* (the killer parasite) to avert its spread and saving lives [11]. Dipsticks (popularly known as rapid test kits) proved boon to the control programme providing diagnosis and treatment at doorstep curtailing inordinate delays between confirmed diagnosis and treatment. Induction and training of health staffs particularly ASHA (accredited social health activists) workers helped improve disease surveillance and prevent morbidity and attributable deaths. All these alternate interventions were accepted by the control programme and duly incorporated in letter and spirit strengthening health systems. Health education measures resulted in improved awareness on disease prevention and control among masses. Communities today stand better informed and extended cooperation, and much needed compliance was forthcoming in keeping with slogan '*Zero Malaria Starts with Me*' taking ownership to accelerate towards elimination.

Assam today stands out to be the major beneficiary state with minuscule of cases from NE region and fast approaching pre-elimination stage reporting <500 cases in 2020 and counting [12]. Disease transmission trends are clearly deaccelerating each passing year, yet there are multiple challenges to defeat malaria, viz., asymptomatic malaria (that remains largely unattended there being no surveillance mechanism and treatment policy to mitigate the sea of parasite reservoir), and inadequate population coverage of interventions that are tantamount to achieve elimination [13].

Given in the following chapters is an illustrated account of research activities during the period (1988 – 2016), data collection and analyses specific to NE India helping understand current disease epidemiology for benefit of young researchers, stake-holders and non-governmental organizations (NGOs) to address the gaps of information. This compendium of observations would be of immense value to programme managers helping formulate policy that is community-based, eco-friendly, doable, and self-sustainable to 'end malaria' in the NE region of India. Conquering malaria in NE (a region of strategic significance) will help pave the way to attain the set goal of malaria-free status in the country by the target date of 2027.

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2

North-East India: topography, populations, climate, and the malarial receptivity

North-East (NE), an easternmost region of India (22^{0}N – 29^{0}5′N latitude and 88^0 E - 97^{0}30′E longitude) is of strategic significance in many ways: (i) for its geographical proximity to neighbouring countries sharing vast international border (about 99% of its total geographical boundary) with Tibet Autonomous Region of China to the north, Myanmar to the east, Bhutan to the west and Bangladesh to the south, (ii) considered to be one of the largest panhandles in the world for its connectivity to mainland India only by a narrow strip (chicken's neck), (iii) inhospitable and treacherous terrain, (iii) incessant rainfall and floods coupled with hot and humid climate, and (iv) scores of indigenous tribes rich in cultural diversity (rare human gene pool). What was commonly believed to be Assam, presently comprises seven contiguous sister states namely Arunachal Pradesh (formerly known as NEFA), Assam, Meghalaya, Manipur, Mizoram, Nagaland and Tripura; beginning 2002, state of Sikkim (geographically apart) was also integrated in the North-Eastern Council (NEC) for administrative and logistics purposes (Figure 1).

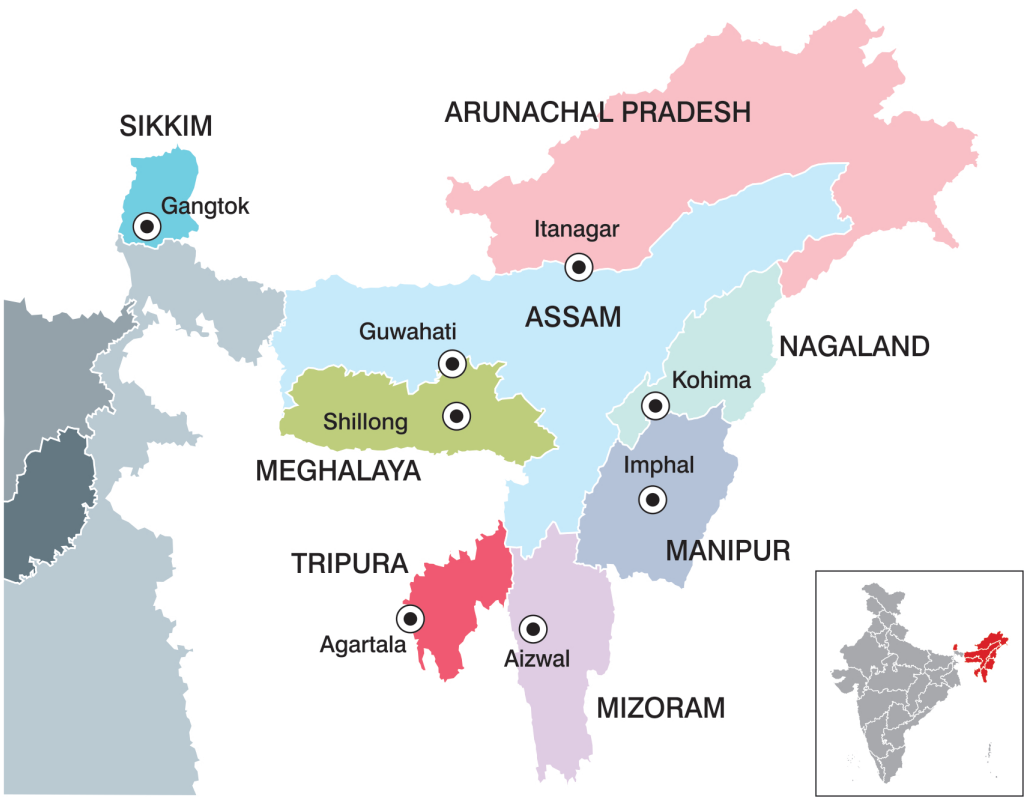

Figure 1. Geographical proximity of the north-eastern states including Arunachal Pradesh, Assam, Manipur, Mizoram, Meghalaya, Nagaland, Tripura and Sikkim. Encircled dots denote location of state capitals. Inset is Map of India showing geographic location of north-east region marked in red (entire north-east region is connected by a narrow stretch between mainland India and Bangladesh). Source: Wikipedia

NE is a vast geographical area (2,62,230 sq. kms) comprising about 8% of India's landed territory harbouring huge evergreen rainforest rich in fauna and flora. Populations are diverse and sparse characterised by regional languages, dialects, costumes, and ritual practices [1]. Terrain is varied ranging from lush-green valleys, foothills, and mountainous regions with elevations ranging from almost sea level (~100 feet above msl) to Himalayan ranges (23,000 feet above msl). In most parts, it rains heavily (1.5 - 3 metres) receiving mostly from southwest monsoons commencing April up until September (nearly six months); flash-floods are annual events leaving many villages/blocks marooned restricting access to essential supplies and healthcare. The region has predominantly sub-tropical climate with hot and humid summers and mild winters in the valleys, and cooler environs up in the hills with rising elevations and that of alpine up in the mountainous regions >6500 feet above msl. Humidity is high (>70%) for most part of the year and water resources are abound marked with major river systems and tributaries. The meteorological data for the representative year of typical NE state of Tripura with majority populations living in valleys and foothills is presented in Table 1.

Table 1. Meteorological data of the state of Tripura, North-East India of 2012*

Month (2012)	Rainfall (mm)	No. of rainy days	Temperature (^{0}C)		Average Relative Humidity (%RH)
			Max	Min	
January	7.2	1	24.3	11.7	79
February	1.2	0	29.2	12.7	63
March	2.0	0	33.4	21.6	60
April	242.1	13	32.7	23.1	71
May	182.4	11	34.2	24.6	73
June	401.0	16	32.1	26.1	82
July	353.7	16	32.2	25.9	81
August	337.0	13	32.8	25.9	81
September	211.4	12	32.8	25.8	83
October	63.7	6	32.1	23.0	80
November	39.2	4	28.9	17.4	81
December	1.4	0	23.6	12.3	89

*India Meteorological Department, Meteorological Centre, Agartala, Tripura

Amongst NE states, Arunachal Pradesh is the largest state with vast forest reserve inhabited by indigenous tribes distributed sparsely in hills and valleys elevations ranging from foothills to Himalayan snow-capped peaks (Table 2). Assam is the second largest state having highest population density mostly living in valleys and foothills. Tripura instead is small state sharing vast international border (84% of total border length) with Bangladesh having large population living in forest-fringe border villages. All other states namely Meghalaya, Manipur, Mizoram and Nagaland are hilly interspersed with plains and valleys. Soil is alluvial rich in humus and naturally fertile supporting host of crop species. Paddy (jhoom – shifting cultivation) is the major occupation others included handlooms, sericulture, horticulture, and forest produce for self-subsistence. NE, considered as

biodiversity hotspot, is home to several medicinal plant species many of which are endemic to this region. Most states have >60% of the land area under forest cover rich in faunal diversity. Tea cultivation is the major industry (mostly in Assam) supplemented by rubber plantation, coal and oil reserves, and timber. The region is prone to seismic activity revisited by earthquake jolts ranging anywhere between 3^0 - 6^0 on Richter scale annually.

Table 2. Demographic characteristics of the North-Eastern states of India

State (Statehood year)	Coordinates	Area sq. km	Population (2019)	Population density per sq. km	% Literacy rate	% Forest cover	Elevation range in metres above msl
Arunachal Pradesh (1987)	26.28° - 29.30° N latitude and 91.20° - 97.30° E longitude	83,743	1,674,976	17	67	80	100 - 5500
Assam (1947)	24° 8′ - 28° 2′ N latitude and 89° 42′ - 96° E longitude	78,438	35,498,714	397	86	36	50 - 1000
Manipur (1971)	23°83′ – 25°68′ N latitude and 93°03′ – 94°78′ E longitude	22,327	3,372,689	128	77	75	700 - 1800
Meghalaya (1972)	20.1° - 26.5° N latitude and 85.49 ° - 92.52 °E longitude.	22,429	3,680,741	132	76	76	150 - 1961
Mizoram (1987)	21°56′ - 24°31′N latitude and 92°16′ - 93°26′E longitude	21,087	1,285,170	52	91	86	1000 - 2210
Nagaland (1963)	25 - 26.1584° N latitude and 94.5624° - 95 E longitude	16,579	2,063,000	119	80	75	610 - 1800
Tripura (1971)	22°56′ - 24°32′N latitude, and 91°09′ - 92°20′E longitude	10,491	4,169,794	350	95	60	12 -1000

Source: Northeast Wikipedia (https://en.wikipedia.org/wiki/Northeast_India)

In large tracts of northeast, climatic conditions are congenial for mosquito proliferation and longevity facilitating transmission of malarial pathogen [2]. Mosquito fauna is rich and breeding sources are numerous supporting efficient carriers of malaria parasite [3]. Among six dominant malaria vector species in India, *Anopheles minimus* and *An. baimaii* (formerly known as *An. balabacensis* and later *An. dirus*) reckoned as most efficient for strong predilection for human host, are widely prevalent and incriminated [4]. All north-eastern states (except Sikkim reporting imported cases) are malaria endemic having perennial and persistent transmission contributing significant number of cases in relation to population share to India's country total reported cases annually. Drug-resistant malaria was detected first in Assam for which NE is believed to be the epicentre and corridor for spread to peninsular India and beyond [5].

In most parts of NE, cases are recorded throughout the year with seasonal peak corresponding to months of rainfall (April – September); for remainder of the season cases were recorded but far less in number [6]. There are records of devastating focal disease

outbreaks across its landscape adding penury to the poverty-stricken marginalized population groups mostly living in forest-fringe villages bordering interstate and/or those sharing international border [7]. During Second World War (1939-1945), causalities due to malaria far exceeded than those warring forces against Japanese aggression in Manipur/ Nagaland [8]. Heaps of dead bodies were observed attributed to malarial fever, and much of the resources were invested in preventing malaria in troops.

Both *Plasmodium falciparum* and *P.vivax* occurred in abundance [9], however, parasite formula and regional distribution varied between states (Figure 2). Of these, while *P. vivax* predominated in cooler climates including states of Arunachal Pradesh and Nagaland (61% – 80% of total cases); *P. falciparum* was the majority infection (≥70%) in Assam, Meghalaya, Mizoram and Tripura. Instead, in Manipur, both parasite species occurred in intermediate proportions ranging from 31 – 60 per cent.

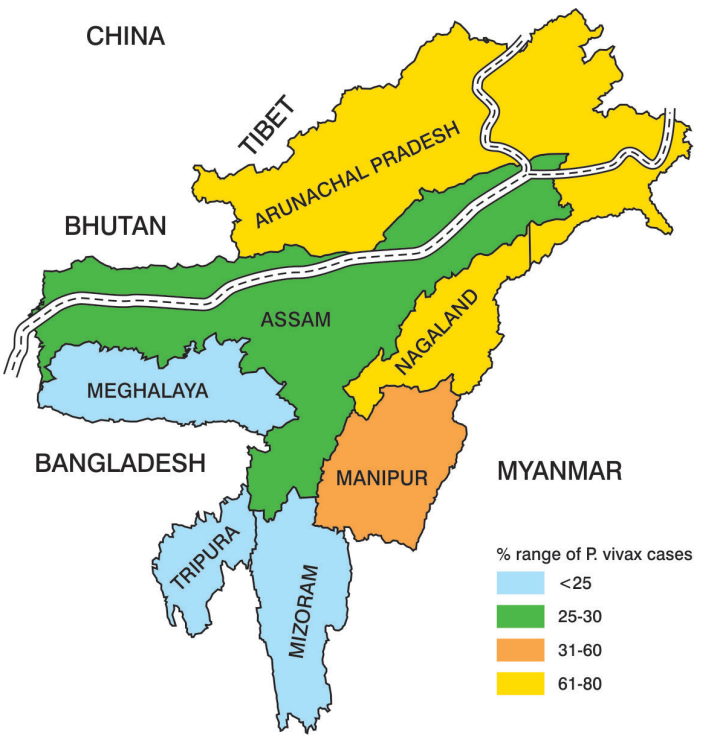

Figure 2. Distribution of *Plasmodium vivax* cases in the North-Eastern states of India for data based on 2016-2019. International borders are demarcated by bold line and state boundaries are colour coded showing relative abundance of *Plasmodium vivax* (% of total malaria reported cases). Data source: National Vector Borne Disease Control Programme, India.

During 2014 – 2019, cumulatively just 4% population of north-eastern states contributed 4 –12 per cent of reported cases (90% of which were *P. falciparum*), and deaths even higher (solely attributed to *P. falciparum* infection) ranging anywhere from 12 – 39 per cent of those reported in India (Table 3). The actual disease burden is estimated to be manifold for disease surveillance what can be best described as grossly inadequate and for cases not captured from private and public sector alike. Besides there is sea of asymptomatic reservoir (estimated to be anywhere from 10% - 30% of endemic populations) for which

there exists no mechanism for detection least the treatment [10]. Nevertheless, the rollout of newer interventions, viz., insecticide-treated netting materials (ITNs) and artemisinin-based combination therapy (ACT), has resulted in substantial transmission reduction in the NE states. Cases are presently on the decline; however, *P. falciparum* (the killer parasite) continued to be the predominant infection which has evolved from mono-to-multidrug resistant (Figure 3).

Table 3. Relative contribution of malaria cases from the North-Eastern states of India*

Year	No of malaria cases		No. of *Plasmodium falciparum* cases		No. of malaria-attributable death cases	
	Total reported cases in India	Cumulative data from north-eastern states (% share contribution)	Total reported cases in India	Cumulative data from north-eastern states (% share contribution)	Total reported cases in India	Cumulative data from north-eastern states (% share contribution)
2014	1102205	136256 (12)	722546	122152 (17)	562	222 (39)
2015	1169261	132109 (11)	778821	112544 (14)	384	135 (35)
2016	1087285	65180 (6)	711502	54274 (8)	331	76 (23)
2017	844558	36521 (4)	529530	30155 (6)	194	23 (12)
2018	429928	28335 (6)	207198	25642 (12)	96	24 (25)
2019	338494	25229 (7)	156940	22918 (14)	77	17 (22)

*Source: National Vector Borne Disease Control Programme

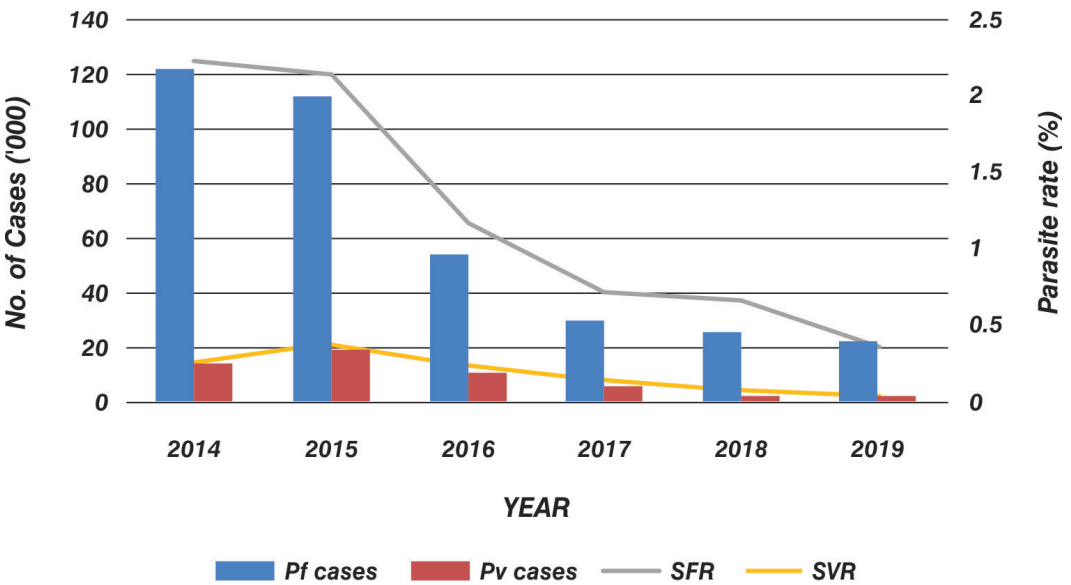

Figure 3. Reducing trends of malaria transmission in the North-Eastern states based on cumulative data of the seven sister states, including Arunachal Pradesh, Assam, Meghalaya, Mizoram, Nagaland, Manipur and Tripura (2014-2019). SFR (smear falciparum rate) and SVR (smear vivax rate) denote % blood-smears positives for malaria parasite: Pf = *Plasmodium falciparum*, Pv = *Plasmodium vivax*. Data source: National Vector Borne Disease Control Programme, India.

In the NE region, up until turn of the millennium, Assam alone (66% of the total population of the NE region), carried brunt of the disease burden contributing >50% of total reported cases (Table 4). However, in the past decade or so, Assam has made major gains in containing the disease onslaught by mobilizing resources both from domestic as well as Global Fund to fight Aids, Tuberculosis and Malaria (GFATM) for strengthening interventions against vector as well as parasite which paid rich dividends in reducing malaria cases and deaths [11]. Nevertheless, transmission continued unabated in states of Meghalaya, Mizoram and Tripura for lack of adequate interventions in place and time, cumulatively the trio constituted bulk of cases (90%) most of which were due to *P. falciparum* (Table 5).

Table 4. Comparative epidemiological data on malaria transmission in the North-Eastern states of India for data based on 1999*

State	No. of smears +ve for malaria		% smears +ve for *Plasmodium falciparum*	Malaria attributable death cases
	Plasmodium falciparum	*Plasmodium vivax*		
Arunachal Pradesh	10263	47980	18	01
Assam	83064	47984	63	111
Manipur	1399	1263	53	08
Meghalaya	9153	5645	62	05
Mizoram	9575	4862	66	73
Nagaland	202	4194	05	12
Tripura	11889	12519	83	11
Sikkim	02	12	14	0

*Source: National Vector Borne Disease Control Programme

It is amply clear that strengthening interventions in these three states (comprising 17% of total population of NE contributing 90% of cases), should be the topmost priority. These states share vast international border with Bangladesh calling for stronger cross-border initiative for coordinated interventions mitigating malarial threat. Reported cases in Arunachal Pradesh are mostly concentrated in foothill villages bordering Assam attributed to inadequate interventions related to cross-border malaria. In Manipur, cases were the least probably due to grossly inadequate disease surveillance and reporting. Assam (the entry point to northeast India) instead has made impressive gains and reporting steady decline in cases each passing year. Malaria transmission is de-accelerating in the state evidenced by record fall in cases from 131,048 in 1999 to just 1459 in 2019 (99%) reaching almost the pre-elimination levels. It is an exemplary accomplishment in a state what was once considered invincible. The road to success has been long and winding spanning over 20 years of research in high-risk pockets but well worth the efforts yielding sustainable solutions to long-standing health problem. The approach was based on three main pillars including: (i) understanding local disease epidemiology and transmission dynamics, (ii) field-evaluation of newer interventions both against disease vectors and the parasite, (iii) health education and awareness eliciting community participation and enhanced compliance. The task was arduous but was made possible by systematic research and innovation of evidence-based technologies enabling decisive attack on disease vectors and parasite, and above all developing human resource helping strengthen healthcare services in reaching out most at risk.

Table 5. Comparative epidemiological data of malaria transmission in the North-Eastern states of India during (2016-2019)*

State	Year	Pop. (millions)	No. of malaria cases	No. of *Plasmodium falciparum* cases (% of total cases)	Annual parasite incidence (No. of cases per 1,000 pop.)	No. of malaria - attributable death cases
Arunachal Pradesh	2016	1.57	3,128	895 (29)	1.99	2
	2017	1.60	1,546	488 (31)	0.95	0
	2018	1.63	625	154 (25)	0.37	0
	2019	1.67	139	27 (19)	0.08	0
Assam	2016	33.90	7,826	5,686 (73)	0.23	6
	2017	34.49	5,281	3,494 (66)	0.15	0
	2018	35.01	3,816	2,859 (75)	0.11	2
	2019	35.60	1,459	872 (60)	0.04	4
Manipur	2016	2.97	122	58 (47)	0.04	0
	2017	3.21	80	22 (27)	0.02	0
	2018	3.27	12	3 (25)	0.003	0
	2019	3.37	16	5 (31)	0.004	0
Meghalaya	2016	3.21	35,147	31,867 (91)	10.95	45
	2017	3.47	16,454	14,418 (88)	4.74	12
	2018	3.53	6,394	6,065 (95)	1.81	6
	2019	3.68	2,615	2,364 (90)	0.71	4
Mizoram	2016	1.20	7,583	5,907 (78)	6.07	9
	2017	1.23	5,715	4,974 (87)	3.78	4
	2018	1.26	4,296	3,937 (92)	2.72	3
	2019	1.28	8,543	8,010 (94)	6.67	8
Nagaland	2016	2.98	828	316 (38)	0.28	0
	2017	3.12	394	188 (48)	0.13	1
	2018	3.20	113	24 (21)	0.03	0
	2019	3.27	20	4 (21)	0.006	0
Tripura	2016	3.94	10,546	9,545 (90)	2.68	14
	2017	4.42	7,051	6,571 (93)	1.59	6
	2018	4.54	13,079	12,600 (96)	2.88	13
	2019	4.68	12,437	11,636 (94)	2.66	1

*Source: National Vector Borne Disease Control Programme, India

To maintain productivity, containing infectious diseases in the NE should be the utmost priority for harmonious growth across strata. Among these, malaria has been in the forefront which has plagued the communities for decades hampering the equitable socio-economic development. Defeating malaria in northeast India is of paramount importance not only to prevent spread of drug-resistant strains but also which could prove to be the forerunner for achieving malaria elimination in the country [12]. In the foregoing chapters, the problem of malaria in Assam and approach for its containment are the focus of attention which could serve as ready reckoner for other endemic territories to end transmission for good in keeping with global agenda for malaria-free world by 2030.

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Mosquito Fauna and Vectors of Malaria

3

Anopheles mosquito fauna and breeding habitats: targeting interventions for vector control

Introduction

India is a vast country with varied ecology and climatic zones having implications in malaria transmission and vector control strategies [1]. Disease epidemiology is complex with multiplicity of disease vectors and varied transmission receptivity across its landscape. Of total 465 *Anopheles* species occurring globally, 61 species are reported to occur in India split into subgenera *Anopheles* and *Cellia* [2]. Ever since initial faunistic surveys by Christophers [3] and Puri [4], lot more information has been added related to species groups (closely related but distinct species) prevalent in India and taxonomic keys have been updated substantially supplemented by illustrations facilitating easy identification [5-7]. Aided by molecular tools, many species of the subgenus *Cellia* have been characterized to be sibling-species complexes (morphologically undistinguishable, but partially or completely reproductively isolated by pre-mating or post-mating barriers), and more are being unravelled [8-9]. As many 12 species belonging to various species complexes are implicated in malaria transmission in the Indian subcontinent [2], of which six are considered dominant of epidemiological significance. These included sibling-species of the *An. culicifacies* s.l., *An. fluviatilis* s.l., *An. minimus* s.l., *An. dirus* s.l., *An. sundaicus* s.l. and *An. stephensi* spread in different physiographic risk zones of India [10,11]. Amongst malaria-endemic regions, North-East (NE) India is most critical for proportionally larger share of cases and spread of drug-resistant malaria [12]. It is rich in biodiversity with huge rainforest reserve ranging anywhere from 40% – 80% of the total land area associated with favourable climatic conditions encompassing diverse ecology, numerous breeding habitats and year-round high humidity (60%- 80%). Nevertheless, over the past decade NE has witnessed rapid transformation associated with economic boom and infrastructure development, urbanization, rail, road and air connectivity at expense of deforestation and population migration across borders resulting in altered ecology. Faunistic changes are happening with ecological succession of new vector species, hitherto, occurring in low density [13]. It is, therefore, imperative to ascertain *Anopheles* mosquito fauna aiming prevalence and incrimination of vector species helping target species-specific interventions for containment. This chapter includes an account of reported *Anopheles* species and their bionomics including information on seasonal abundance and breeding preferences in a typical malaria-endemic block of Assam (the economic corridor to NE India).

Anopheles mosquito fauna diversity

Year-round mosquito collections (January – December 1988) were made by hand-catch method using suction tube aided by battery torch light from human dwellings (indoor-resting) and cattle-biting during early morning (05:00 – 07:00) and evening (18:00 – 20:00)

hrs respectively in a malaria-ridden block of district Kamrup, Assam, NE India. A typical human dwelling unit included house made of split-bamboos (permitting entry/exit and easy access of mosquitoes to human host) and cattle-shed with thatched roof in close vicinity (Figure 1).

Figure 1.
A typical human dwelling in a malaria-ridden village of Assam with house made of split-bamboo and closely annexed cattle-shed. Hordes of mosquitoes were seen resting on thatched roofing and feeding on cattle during evening collections after dusk.

Cumulatively for all seasons, 23 *Anopheles* mosquito species occurring in varied proportions were collected from cattle-sheds and human dwellings indoors and identified (Table 1). Of these, *An. nivipes* (formerly identified as *An. philippinensis*) was the most predominant species constituting bulk of anopheline fauna (29.94% of total collections). The other common species included *An. vagus* (18.80%), *An. splendidus* (16.65%) and *An. nigerrimus* (14.72%). Among others, *An. kochi*, *An. minimus*, *An. annularis* and *An. aconitus* constituted fair proportion of the fauna in decreasing order; the remaining constituted <1% of the total collections. Majority of these mosquito species were collected resting/feeding in cattle-sheds after sunset, while *An. minimus*, *An. vagus*, *An. varuna* and *An. fluviatilis* s.l. were most abundant in human dwellings indoors in early morning resting collections before sunrise. Inside the human dwellings, most of these species were seen resting on the walls, hanging clothes, underneath cots, and miscellaneous bamboo/wooden furniture items in darker/shady corners of the house clearly avoiding sunlit areas. Mosquito collections using CDC miniature light trap (Figure 2) yielded similar results for prevalence of *Anopheles* species, irrespective of the ecotype, but not truly representative of their seasonal abundance (Table 2).

Seasonal abundance and mosquito biting rates

Anopheles mosquito species were prevalent during all seasons in various ecotypes, but their densities were seen rising concomitant with rising temperatures and onset of rainfall beginning April/May and was high (range 47 – 74 per person hour) during June - October corresponding with the wet season (Figure 3). This peak, however, was largely attributed

Table 1. Relative abundance of *Anopheles* mosquito species in different biotopes in a typical malaria-endemic block of Kamrup district, Assam, NE India (January – December 1988)

Anopheles species	No. collected (% of total)	Density per person hour	
		Cattle-biting	Human dwellings (indoor day-resting)
An. aconitus Doenitz, 1902	568 (1.72)	0.87	0.12
An. annularis Van der Wulp, 1884	653 (1.97)	1.48	0.24
An. barbirostris Van der Wulp, 1884	228 (0.69)	0.38	0.00
An. baimaii Sallum & Peyton, 2005	30 (0.09)	0.003	0.002
An. culicifacies s.l. Giles, 1901	20 (0.06)	0.08	0.01
An. fluviatilis s.l. James, 1902	12 (0.03)	0.00	0.68
An. jamesii Theobald, 1901	77 (0.23)	0.12	0.00
An. jeyporiensis James, 1902	47 (0.14)	0.18	0.24
*An. karwari (*James*),* 1902	25 (0.07)	0.08	0.00
An. kochi Doenitz, 1901	2291 (6.92)	1.70	0.01
An. maculatus Theobald, 1901	212 (0.64)	0.24	0.06
An. majidi Young & Majid, 1928	2 (0.01)	0.02	0.00
An. minimus Theobald, 1901	1650 (4.98)	0.12	6.58
An. nigerrimus Giles, 1900	4870 (14.72)	6.25	0.01
*An. nivipes (*Theobald*)*, 1903	9910 (29.94)	13.80	0.04
An. pallidus Theobald, 1901	150 (0.45)	0.30	0.00
An. ramsayi Covell, 1927	2 (0.01)	0.06	0.00
An. splendidus Koidzumi, 1920	5507 (16.65)	8.44	0.01
An. subpictus Grassi, 1899	85 (0.26)	0.06	0.00
An. tessellatus Theobald, 1901	213 (0.64)	0.17	0.00
An. theobaldi Giles, 1901	5 (0.01)	0.01	0.00
An. vagus Doenitz, 1902	6221 (18.80)	4.79	6.15
An. varuna Iyengar, 1924	311 (0.94)	0.22	1.52

to rise in densities of *An. nivipes*, the most common species. With the cessation of rainy season, however, a sharp decline in anopheline density was observed commencing November all through February/March (the winter season) during which precipitation was also minimal. *An. fluviatilis* s.l. species, however, was encountered during post-monsoon months only (winter season) and its densities varied from 0.10 – 3.15 per person hour, maximum being during January – April.

Mosquito biting rates (MBR) were ascertained through overnight dusk-to-dawn (18:00 – 05:00 hrs) human-landing catches during April – November 1988 (Table 3). Mean MBR per person night varied from 0.03 for *An. culicifacies* s.l. to 5.82 for *An. minimus*, and cumulatively for all species from 4.00 in November to 43.33 in June. *An. minimus* was the most predominant species and its MBR ranged from 1.00 in April to 15.83 in June (the highest of the season). MBR for *An. aconitus*, *An. nivipes*, *An. varuna*, *An. splendidus*, *An. kochi* and *An. annularis* were 3.47, 2.59, 1.74, 1.32, 1.29 and 1.15 respectively in decreasing order; for all other species it was <1 per person night.

Table 2. CDC miniature light-trap *Anopheles* mosquito collections in human dwellings indoors (18:00 – 05:00 hrs) in a typical malaria-endemic block of Kamrup district, Assam, NE India

Month (1990 – 1991)	May, 1990	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan, 1991	Feb	Mar	Apr	Total trap nights	% of total Mosquito Adults
Traps nights	2	2	2	2	5	5	7	4	5	3	4	3	44	
Anopheles species	No. Adults	No. Adults	No. Adults	No. Adults	No. Adults	No. Adults	No. Adults	No. Adults	No. Adults	No. Adults	No. Adults	No. Adults	No. Adults	
An. aconitus	0	0	1	0	0	2	0	1	1	0	6	4	15	2.16
An. annularis	0	0	0	0	13	2	5	0	1	0	2	11	34	4.89
An. baimaii	0	1	1	3	0	0	0	0	0	0	0	0	5	0.72
An. barbirostris	0	0	0	0	2	1	0	0	0	0	0	0	3	0.43
An. culicifacies s.l.	0	0	0	0	0	0	0	0	0	0	0	3	3	0.43
An. fluviatilis s.l.	0	0	0	0	0	1	2	1	2	0	2	2	10	1.44
An. jamesii	0	0	0	0	0	0	0	0	0	1	0	0	1	0.14
An. jeyporiensis	0	0	0	0	0	6	3	3	2	1	2	3	20	2.87
An. kochi	0	0	0	0	0	12	2	0	0	0	20	3	37	5.32
An. maculatus	0	0	0	0	0	1	1	0	2	0	0	0	4	0.57
An. minimus	4	1	3	0	23	25	25	9	4	4	4	4	106	15.23
An. nigerrimus	5	1	0	0	3	9	5	0	1	0	6	0	30	4.31
An. nivipes	8	0	6	0	45	7	28	0	2	2	72	30	200	28.74
An. splendidus	0	0	0	0	0	1	2	0	0	0	33	4	40	5.75
An. tessellatus	0	0	0	1	0	0	0	0	0	0	0	0	1	0.14
An. vagus	0	1	0	9	36	3	1	2	0	0	47	15	114	16.38
An. varuna	0	0	0	0	0	17	14	7	4	11	7	13	73	10.49
Total	17	4	11	13	122	87	88	23	19	19	201	92	696	100

Figure 2. Application of battery-operated CDC miniature light trap for sampling mosquito adult populations in human dwellings can help ascertain mosquito fauna and supplement mosquito collections in the given locality.

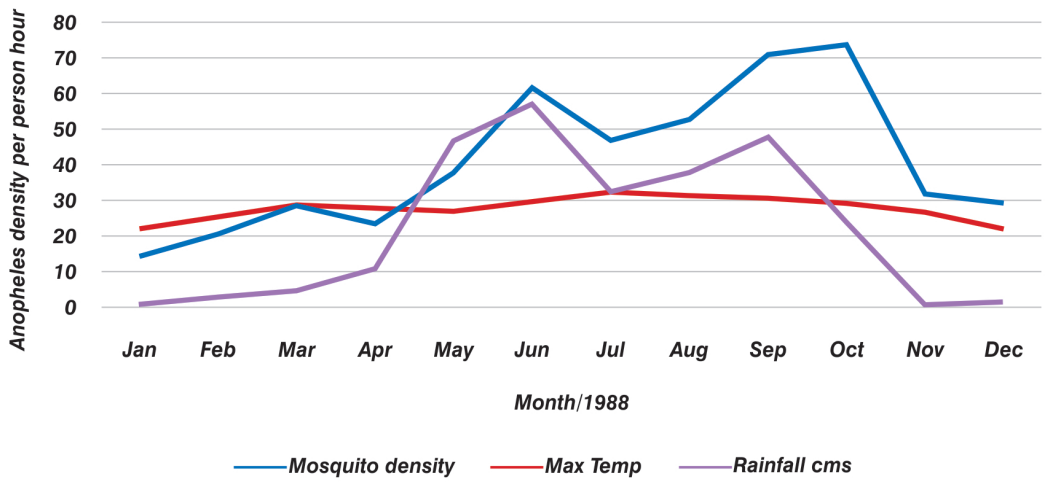


Figure 3. Seasonal abundance of *Anopheles* mosquito species and climatic conditions in the typical malaria-endemic block of Kamrup district, Assam, NE India (January - December 1988). Cumulative mosquito density for all species relates to number of mosquito adults collected per person hour. Rise in temperature (^{0}C) and rainfall were seen critical resulting in build-up of mosquito density.

Breeding habitats

Anopheles mosquitoes were recorded breeding in a variety of habitats including ponds, wells, roadside ditches, perennial streams, shallow pits and paddy fields (Table 4). Paddy fields particularly those at 30 cm of plant growth and after harvest served an ideal ecosystem supporting breeding for bulk of mosquito species most common being *An. nivipes*, *An. nigerrimus*, and *An. vagus* (Figure 4). Similarly, *An. barbirostris* was recorded breeding in all possible habitats except pits and those ready to harvest paddy fields. Slow-flowing perennial seepage water streams hosted virtually all mosquito species except *An. karwari*, *An. pallidus* and *An. tessellatus*. *An. minimus* (the proven vector) were recorded breeding in these streams throughout the year and closely annexed paddy field with perceptible flow of

Table 3. Mosquito biting rate per person night of anophelines in a typical malaria-endemic block of Kamrup District of Assam, NE India

Month (1988)	April	May	June	July	August	September	October	November	Total
Persons x nights	**2 x 1 = 2**	**2 x 2 = 4**	**2 x 3 = 6**	**2 x 2 = 4**	**2 x 2 = 4**	**2 x 3 = 6**	**2 x 3 = 6**	**2 x 1 = 2**	**34**
An. aconitus	2.00	0.50	6.50	0.50	12.00	3.50	0.33	0.00	3.47
An. annularis	0.00	0.25	2.67	1.00	1.50	1.33	0.67	0.00	1.15
An. baimaii	0.00	0.00	0.67	0.00	2.00	0.83	0.17	0.00	0.53
An. barbirostris	0.00	0.00	0.33	0.50	0.00	0.17	0.00	0.00	0.15
An. culicifacies s.l.	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.03
An. jeyporiensis	0.00	0.00	0.17	0.25	0.00	0.83	0.17	0.00	0.24
An. kochi	1.50	0.00	2.33	1.25	1.00	1.33	1.67	0.00	1.29
An. maculatus	0.50	0.00	0.83	0.00	0.00	0.00	0.00	0.00	0.18
An. minimus	1.00	11.25	15.83	5.00	2.25	2.50	1.50	1.50	5.82
An. nigerrimus	0.00	0.00	0.17	0.00	0.00	0.50	1.33	0.00	0.35
An. pallidus	0.00	0.25	0.33	0.00	0.00	0.17	0.00	0.00	0.12
An. nivipes	0.50	0.00	2.67	1.00	3.25	4.50	4.33	0.50	2.59
An. splendidus	0.00	0.25	4.83	1.25	0.75	1.00	0.17	0.00	1.32
An. subpictus	0.50	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.06
An. tessellatus	0.00	0.00	0.50	0.25	0.00	0.00	0.00	0.00	0.12
An. vagus	2.00	0.25	2.83	0.00	0.00	1.00	0.00	1.00	0.88
An. varuna	3.00	0.25	2.50	0.25	5.25	0.83	1.33	1.00	1.74
Total	**11.00**	**13.00**	**43.33**	**11.50**	**28.00**	**18.50**	**11.67**	**4.00**	**20.03**

water. Breeding of *An. vagus*, a species of common occurrence, was recorded in all possible habitats. Instead, *An. pallidus*, *An. culicifacies* s.l. and *An. karwari* were recorded breeding exclusively in ponds, streams and paddy fields respectively. While shallow wells, roadside ditches and pits were positive for a few select mosquito species, good number of mosquito species were recorded breeding in ponds except *An. culicifacies* s.l., *An. jeyporiensis*, *An. karwari*, *An. minimus* and *An. tessellatus*.

Mosquito species groups and sibling-species complexes

Anopheles mosquito species described in India belonged exclusively to subgenera *Anopheles* Meigen and *Cellia* Theobald, many of which are very closely related and have been clubbed into groups based on morphological similarities [7]. Many species of these groups have been reported to be prevalent in NE region having varied bionomical characteristics and implications in vector control (Table 5).

In the published literature prior to 1990s, formerly some species of a particular group were identified as single species due to lack of access to updated pictorial keys and adequate expertise, viz., *An. maculatus*; of which as many six member species are presently known

Table 4. Larval breeding habitats of *Anopheles* mosquito species in Kamrup district, Assam, NE India (January – December 1989)*

Anopheles species	Ponds	Wells	Ditches	Streams	Pits	Paddy fields					
						Barren	Saplings	30 cms	>30 cms	Ready to harvest	After harvest
An. aconitus	+	-	-	+	+	-	-	-	-	+	+
An. annularis	+	-	+	+	-	+	-	+	-	-	-
An. barbirostris	+	+	+	+	-	+	+	+	+	-	+
An. culicifacies	-	-	-	+	-	-	-	-	-	-	-
An. jamesii	+	-	-	+	-	-	-	-	-	-	+
An. jeyporiensis	-	-	-	+	-	-	-	-	-	-	+
An. karwari	-	-	-	-	-	-	-	+	-	-	-
An. kochi	+	-	+	+	-	+	+	+	-	+	+
An. maculatus	+	-	-	+	+	-	-	+	-	-	+
An. minimus	-	-	-	+	-	-	-	+	-	+	+
An. nigerrimus	+	+	+	+	-	+	+	+	+	+	+
An. nivipes	+	-	-	+	+	+	+	+	+	+	+
An. pallidus	+	-	-	-	-	-	-	-	-	-	-
An. splendidus	+	-	+	+	-	-	-	+	-	+	+
An. tessellatus	-	-	-	-	-	-	-	+	-	-	+
An. vagus	+	+	+	+	+	+	+	+	+	+	+
An. varuna	+	-	+	+	+	+	-	+	-	+	+

*(+) denotes presence of larval breeding in the given habitat.

Figure 4. Human dwellings amidst paddy fields in a malaria-endemic village of Assam provide an ideal ecosystem for mosquito proliferation. Water-logged paddy fields at various stages of plant growth and seepage water streams are preferred breeding habitat for several *Anopheles* species. Human settlements with closely annexed cattle-sheds provide easy access for host-bloodmeal.

Table 5. Prevalent species group and member species of genus *Anopheles* in India

Subgenus *Anopheles*	Subgenus *Cellia*
aitkenii group*: aitkenii, bengalensis, insulaeflorum, pinjaurensis*	***annualris*** group*: annularis, nivipes, pallidus, philippinensis*
asiaticus group*: annandalei, interruptus*	***maculatus*** group*: maculatus, pseudowillmori, willmori, dravidicus, rampae, sawadwongporni*
barbirostris group*: ahomi, barbirostris, barbumbrosus*	***minimus*** group*: aconitus, minimus, varuna*
culiciformis group*: culiciformis, sintoni*	
hyrcanus group*: argyropus, crawfordi, nigerrimus, nitidus, peditaeniatus, sinensis*	
lindesayi group*: gigas, lindesayi*	
umbrosus group*: roperi, umbrosus*	

Source Reference [7]

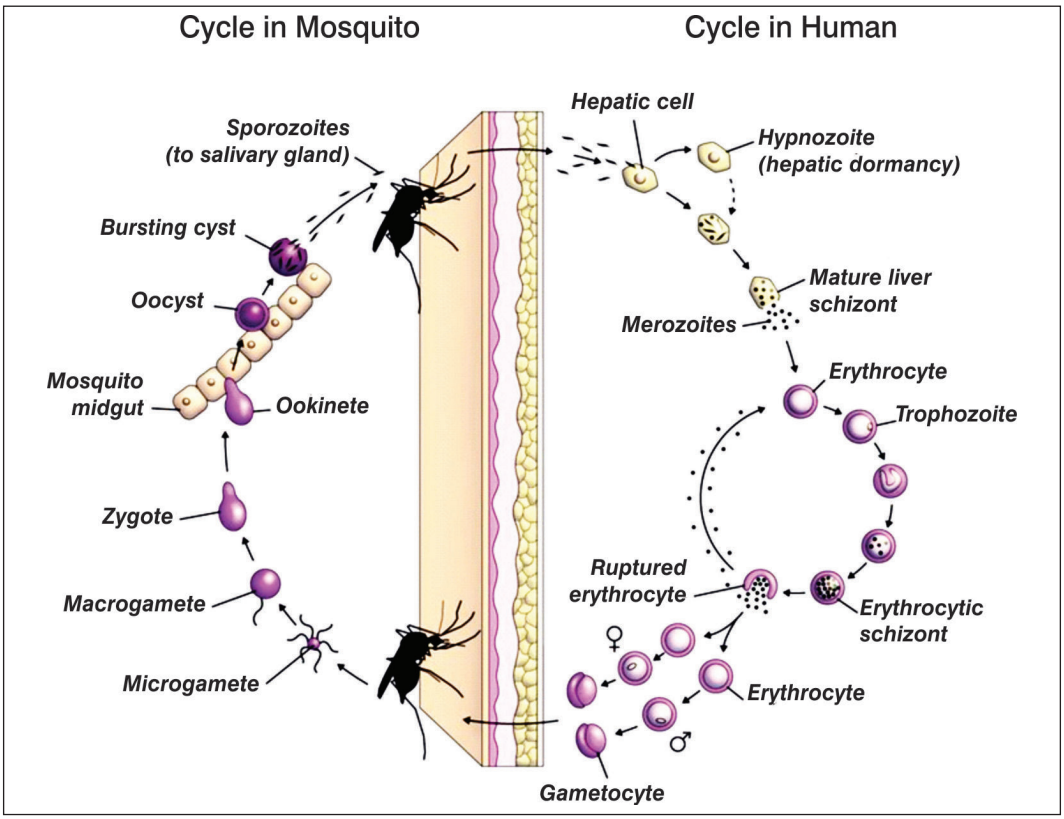
to be prevalent in NE India [14]. Similarly, previously described populations of *An. philippinensis* now have been characterized to comprise both *An. philippinensis* as well as *An. nivipes* in varying proportions [15]. Formerly, all member species of the *An. hyrcanus* group were invariably referred either *An. hyrcanus* or *An. nigerrimus*. Among others, *An. minimus* and *An. dirus* (the predominant vector species), along with *An. culicifacies*, *An. fluviatilis*, *An. annularis*, *An. subpictus* and *An. sundaicus* of the subgenus *Cellia* have been characterized to be species complexes having varied number of sibling-species and distribution range [16].

Vector incrimination and host preferences

Malaria parasite cycle is complex involving multiple stages in two different hosts involving anopheles vector mosquito and human (Box). Mosquitoes are the definitive hosts for the malaria parasites, wherein the sexual phase of the parasite's life cycle (sporogony) is completed resulting in development of innumerable sporozoites which are injected in humans by mosquito bite initiating asexual cycle (schizogony) causing clinical paroxysm. Not all mosquito species are capable of harbouring malaria parasite except a few (vectors) which serve as host and act as carriers of infective stage, the sporozoites. To incriminate mosquito species which is/are vector(s) in a given locality, anopheles adults collected from various biotopes including whole night (dusk-to-dawn) human-landing catches and day-resting indoor morning collections in human dwellings were dissected for gut infections (presence of oocysts on midgut epithelium) and salivary glands for detection of motile sporozoites (the infective stage of malaria parasite). Most species were sporozoite negative except *An. minimus*, *An. fluviatilis* s.l. and *An. baimaii* (formerly identified as *An. dirus*/*An. balabacensis balabacensis*) [10]. Besides these, *An. philippinensis*/*nivipes*, *An. annularis*, *An. aconitus*, *An. maculatus* s.l., *An. subpictus* s.l. and *An. jeyporiensis* are also believed to play some role in malaria transmission with records of occasional sporozoite infectivity [17].

Among *Anopheles* mosquito host species, most were zoophilic having fed on cattle, whereas *An. minimus* and *An. baimaii* and *An. fluviatilis* s.l. were predominantly anthropophilic for

Box: Life cycle of malaria parasite in Mosquito (sporogony) and Human (schizogony)



Source: Malaria site (https://www.malariasite.com/life-cycle/)

having strong predilection for human host [18]. Of these three anthropophilic species, *An. minimus* and *An. baimaii* were repeatedly incriminated across malaria-endemic districts of NE India and are proven efficient vectors requiring targeted interventions for effective vector management [10]. While detection of sporozoite antigen in adult females by immunological techniques is strong indicator of possibility of presence of sporozoites, but detection of motile sporozoites on dissection of salivary glands of field collected mosquito individuals is exclusive evidence of species being a vector of malaria (Figure 5). The detailed bionomical characteristics of these species are presented in the following chapter helping target species-specific interventions.

Ecological succession and implications in vector control

Population explosion, infrastructure expansion, deforestation, urbanization, intensified residual insecticide spray operations and associated contextual determinants have indeed impacted faunistic changes [13]. Consequent to focussed DDT spray operations during 1960s, *An. minimus* (the predominant vector species) was once believed to have disappeared and

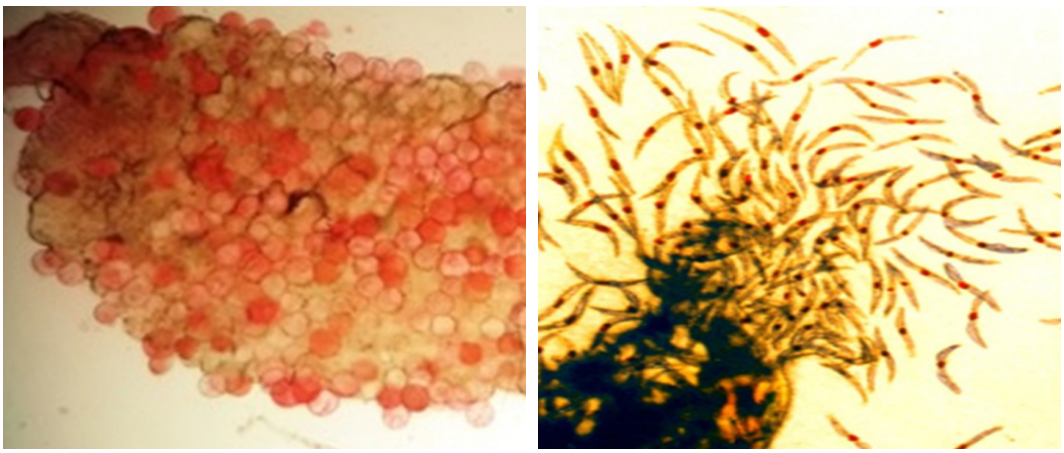


Figure 5. Malaria parasite development in the mosquito host. Left: Mosquito midgut loaded with oocysts (Courtesy: SK Ghosh); Right: Malaria sporozoites (spindle shape nucleated) liberated from mature oocysts making their way into salivary glands (Source: fiickr.com. Credit Electic Manna, NIAID).

An. philippinensis was implicated in disease transmission [19]. *An. minimus* had resurfaced again in 1980s and were recorded resting indoors in human dwellings with its bionomical characteristics intact and held responsible for fulminating disease outbreaks reported across NE India [20]. However, with the rollout of newer intervention tools, long-lasting insecticidal nets (LLINs) in particular, populations of *An. minimus* are once again reported depleting [21]. Instead, *An. culicifacies* (formerly occurring in low densities) is establishing its foothold across NE India occupying niche of *An. minimus*. *An. culicifacies* is fast expanding its range of distribution occupying diverse habitats and assessed to be multi-insecticide resistant threatening malaria elimination efforts [22]. Similarly, populations of *An. baimaii* (forest dweller) are diminishing in their erstwhile domains of distribution associated with the depleting forest cover and increased acreage under cultivation. In addition, populations of several mosquito species, viz., *An. majidi*, *An. ramsayi*, *An. theobaldi* formerly recorded in low numbers presently are fast disappearing, whereas *An. subpictus* (an emerging vector) is diversifying and expanding its territory [13,23]. Nonetheless, as per current records of observations, *An. stephensi* (urban vector), which has already invaded the African horn [24], has not yet been reported to occur in NE India. With growing urbanization, however, it is likely that *An. stephensi* would also invade NE India as well opening new vistas for containment of urban malaria which presently is non-existent. Continued urbanization, increased travel and connectivity have already resulted in invasion of dengue (formerly free of the virus), vectors of which are recorded proliferating in urban and suburban town areas of NE India, now here to stay for long [25,26].

Conclusions

Even though mosquito fauna surveys based in Assam/other states of NE India have been extensive but certainly not exhaustive for lack of data on outdoor resting mosquito species - a paradigm shift that is considered emerging [27]. Although, various applied sampling devises yielded similar results, yet existence of additional *Anopheles* mosquito

species cannot be ruled out in areas not yet explored especially inter-country border pockets [28]. There are records of some species, viz., *An. ahomi* in districts of upper Assam but not recorded to occur lower in the valley [13]. *An. maculatus*, formerly believed to comprises of two varietal forms has now been characterized to encompass six formally recognized species specific to NE India [14]. Similarly, what formerly regarded as *An. fluviatilis*, now has been characterised to be hyper-melanic variant form of *An. minimus* prevalent in winter season [29]. Seasonal spot-surveys in other NE Indian states revealed similar fauna, but relative proportions varied in relation to available breeding sources [30-36]. There are records of additional species, viz., *An. gigas* prevalent in Arunachal Pradesh (the foothills of eastern Himalayas), Nagaland, Manipur and districts of upper Assam[30-33], but not recorded in lower Assam [12]. Faunistic changes are happening inadvertently; there is every likelihood that mosquito species of secondary importance may assume greater role in disease transmission in the elimination era [37]. Vector control is central to contain transmission and should be prioritized by strengthening entomological capacity not only at the national but also at the regional/zonal level [38,39]. From the resting and breeding habitats of vector populations, it is advocated that interventions should be targeted selectively, i.e., (i) human dwellings indoors to check adult mosquito vector populations, (ii) seepage-water streams, the breeding habitat of vector mosquito for anti-larval operations for effective vector management. In this era of malaria eradication, there is an imminent need for building capacities for regular entomological surveillance helping formulate strategies for vector control in place and time to contain transmission and spread of drug-resistant malaria [40].

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4

Vectors of malaria in North-East India: bionomics and disease transmission relationships

Introduction

North-East (NE) India is co-endemic for both *Plasmodium falciparum* and *P. vivax* malaria, however, the former parasite species is the most predominant infection constituting 90% of the reported cases, the remaining are *P. vivax* cases [1]. All seven NE states comprising Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram Nagaland and Tripura have been reporting malaria cases but endemicity and relative proportions of parasite species varied. Given the large rainforest reserve, ambient environs and numerous breeding habitats, mosquito fauna is rich but relative abundance of *Anopheles* mosquito species varied in relation to elevation from sea level and ecology [2]. Disease transmission is perennial and persistent in most parts and cases are recorded up to ~3000 feet above msl [3-5]. Drug-resistant malaria, first recorded in Assam in 1973 [6], is presently widespread and fast emerging multi-resistant threatening malaria elimination [7]. Of 27 *Anopheles* species recorded in the NE region [2], *Anopheles minimus* and *An. baimaii* (sibling-species of the *An. dirus* complex) are most abundant and proven to play major role in malaria transmission [8, 9]. Besides these two vector species of primary significance, *An. fluviatilis* (presently considered variant of *An. minimus*) and *An. culicifacies* were also incriminated with seasonal infectivity [3, 10]. In addition, *An. philippinensis*/*An. nivipes*, *An. varuna*, *An. annularis* and *An. maculatus* constituted fair proportion of the native fauna and considered vectors of secondary importance with records of occasional infections [11, 12]. Over the past decade, wealth of information aided by molecular tools has been generated in understanding insights into malaria vectors and associated transmission intensities in varied ecotypes [13, 14]. Included in this chapter are the salient bionomical characteristics of primary as well as vectors of secondary importance and role they play in disease transmission specific to NE India helping target species-specific interventions for sustainable control.

Primary vectors of malaria

Anopheles minimus, a vector of foothill malaria

What formerly believed to be a single species and invariably documented as *An. minimus* has now been characterized to be a complex comprising of 3 member species, viz., *An. minimus* Theobald., *An. harrisoni* Harbach and Manguin, and *An. yaeyamaensis* Samboon and Harbach distributed in the Southeast Asia [15]. Among these, it is exclusively *An. minimus s.s*. which has been established to be prevalent across NE states and can clearly be distinguished by molecular assays from all other closely related species including *An. varuna* having similar eco-biological characteristics [16]. It is a small sized mosquito with distinct bionomical characteristics and held responsible for bulk of malaria cases (estimated 50% of total reported in NE) [8]. *An. minimus* was believed to

have disappeared from NE India in 1960s due to intensified DDT spray operations, and *An. philippinensis* was implicated in ongoing transmission [17]. However, systematic research efforts post re-emergence revealed that *An. minimus* mosquitoes had staged comeback and were recorded to be prevalent in good numbers in malaria-endemic areas of NE India [18]. It is primarily an endophilic species found in dark/shaded corners of the houses (those left unsprayed) in early morning hours resting on roof, hanging cloths, umbrella and underneath furniture/household items, and constituted bulk of indoor-resting mosquito species (Table 1).

Table 1. Dissection records and relative abundance of day-resting *Anopheles* mosquito species in human dwellings indoors in a typical malaria-endemic block of Kamrup district, Assam, NE India (Oct. 1990 – Sept. 1991)

Anopheles species	Abdominal condition*				Total mosquitoes collected	Parity**					Vector incrimination		
	UF	FF	SG	G		NP	1P	2P	3P	4P	No. Dissected	No. gland positive	Infection rate (%)
An. aconitus	0	0	7	4	11	5	6	0	0	0	11	0	0
An. annularis	11	18	27	53	109	41	34	5	0	0	106	0	0
An. culicifacies s.l.	0	0	7	3	10	2	7	1	0	0	10	0	0
An. fluviatilis s.l.	4	75	60	51	190	51	51	12	0	0	186	0	0
An. jeyporiensis	2	30	6	21	59	12	24	3	0	0	59	0	0
An. maculatus	4	1	0	1	6	1	0	0	0	0	6	0	0
An. minimus	12	380	656	590	1638	489	621	210	7	3	1618	58	3.58
An. nivipes	1	3	5	7	16	1	4	1	0	0	11	0	0
An. vagus	0	5	47	120	172	73	84	11	0	0	172	0	0
An. varuna	18	224	201	207	650	170	203	41	0	0	553	0	0

*UF= Unfed, FF= Fully fed, SG= Semi-gravid, G = Gravid **P = Parity (number of oviposition cycles), NP = non-parous, 1P = Uniparous.

It is a perennial species commonly encountered in huts located within a kilometre radius of seepage water streams (preferred breeding habitat) in hills and foothill up to ~3000 feet above msl. Its density, however, was seen building beginning March/April with onset of pre-monsoon showers preceding the peak transmission season and remained high during April – August (Figure 1). These were also the months receiving high rainfall when temperatures were the optimum.

An. minimus has a strong predilection for human host evidenced by host-blood meal analyses (anthropophilic index >90%) as well as dusk-to-down (18:00 – 05:00) human landing catches in high-risk malaria ridden villages. The mosquito biting activity occurred all through the night but was highly pronounced during midnight 00:00-04:00 hours early in the morning. The mosquito biting rates and EIRs (entomological inoculation rates), however, varied between locations representative of low-to-moderate heterogenous transmission [19]. It has been proven unequivocally to be major vector in NE states evidenced by high parity/longevity lasting 2 -3 weeks as well as sporozoite infections in salivary glands practically in all months; on an average ~3% of the *An. minimus* mosquitoes were recorded to be infective [18].

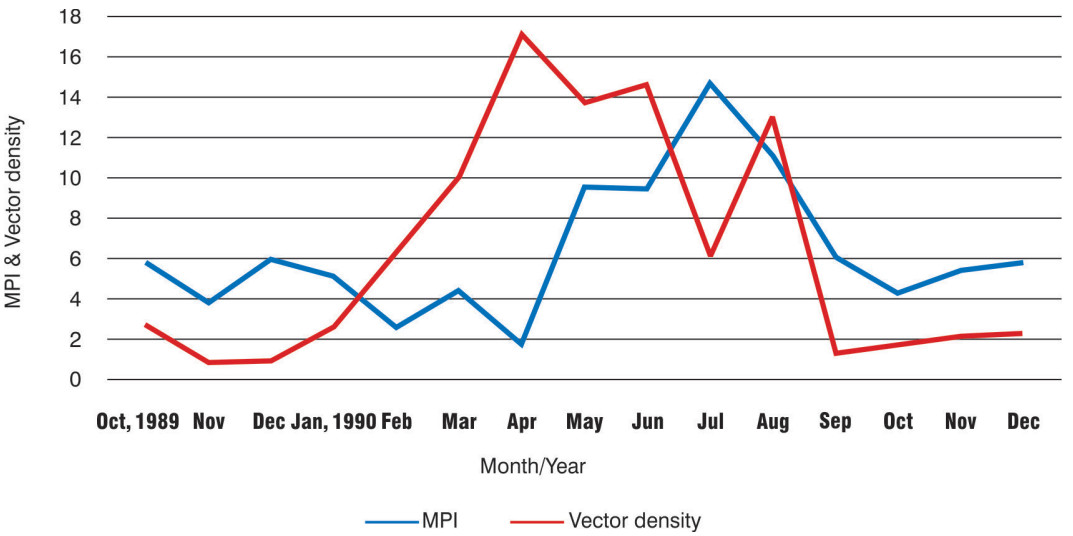

Figure 1. Monthly parasite incidence (MPI) and density of *Anopheles minimus* per person hour in a typical malaria endemic block of Kamrup district, Assam, NE India (Oct 1989 - December 1990).

For control of *An. minimus* vector populations, DDT continues to be in vogue due to its proven susceptibility to this residual insecticide as well as malathion and pyrethroids [8]. Populations of *An. minimus* are once again reportedly getting scarce with the rollout of pyrethroid coated/incorporated long-lasting insecticidal nets (LLINs) but vigil needs to be maintained by continuous monitoring for its innate ability to adapt to varied environs in response to interventions in force [20]. There remains every possibility of its re-appearance even after decades just as that happened in eastern Indian state of Odisha resuming transmission [21].

Anopheles baimaii, a vector of forest malaria

An. baimaii, what formerly identified as *An. balabacensis balabacensis* and later *An. dirus*, is presently confirmed to be exclusively a sibling-species of the *An. dirus* s.l. complex under Leucosphyrus group [16,22]. Sibling-species of the *An. dirus* complex namely *An. baimaii*, *An. cracens*, *An. dirus* s.s., *An. elegans*, *An. nemophilous*, *An. scanloni*, *An. takasagoensis* and a cryptic species *An. aff. takasagoensis* are widespread in Southeast Asia some of which are proven vector in their range of distribution [23]. *An. baimaii* is a medium sized mosquito and morphologically distinct species easily spotted by characteristic broad white-band on tibio-tarsal joint of hind legs and can be unequivocally identified by wide array of molecular assays [9]. It is widespread throughout NE states and commonly encountered in forested/forest-fringed villages (Figure 2) [24]. In such ecotypes, invariably *An. baimaii* is the predominant collection having predilection for human host (Table 2).

An. baimaii is a monsoon species with abundance during wet-season (April – September) inhabiting undisturbed forest reserves and proven efficient vector often linked with transmission of drug-resistant malaria [25]. It is an exophilic species foraging for blood

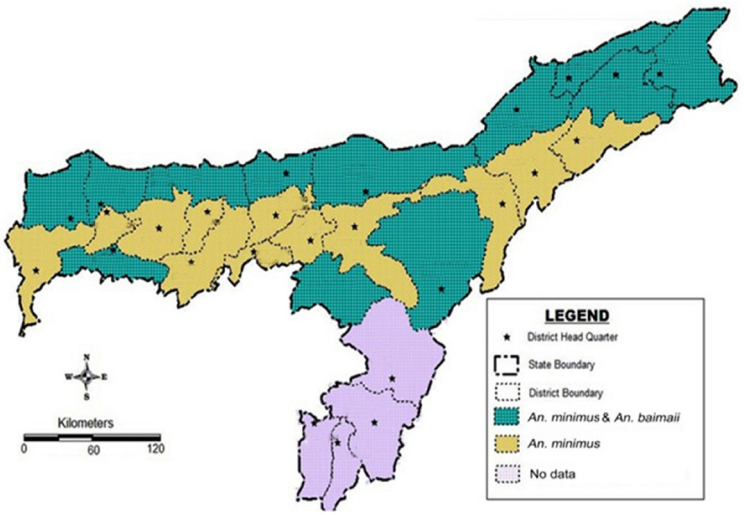

Figure 2. Relative abundance of malaria vectors *Anopheles minimus* and *Anopheles baimaii* in Assam state, NE India (data source:Integrated Disease Surveillance Project, Assam).

Table 2. Relative abundance of *Anopheles* mosquito species in Lawngtlai district of Mizoram (Indo-Bangladesh border), NE India (July – August 2015)

Anopheles mosquito species	Indoor Day-resting (Human dwellings)		Cattle-biting evening collections mosquito density per person hour	CDC Trap (No. Trap nights)		Mean mosquito landing rate per person night (data based on 3 nights)	
	Mosquito density	Total catch		Outdoor (5)	Indoor (4)	Outdoor	Indoor
An. aconitus	0	0	0	2	0	0	0
An. baimaii	0	0	0	1	5	2	1
An. barbirostris	0	0	6	0	0	0	0
An. jamesii	0	0	8	4	0	0	0
An. kochi	0	0	17	3	0	0	0
An. maculatus	0	0	4	2	6	2	1
An. minimus	0	0	0	0	1	0	0
An. nigerrimus	0	0	9	0	0	0	0
An. nivipes	0	0	9	4	0	0	0

meal on human host both indoors and outdoors [26]. It equals *An. minimus* for its high anthropophilic index (>90%) and percent share contribution of cases (mostly *P. falciparum*), and often supplements transmission in conjunction with *An. minimus* during peak season corresponding to months of rainfall resulting in fulminating disease outbreak [27]. This species bites throughout the night, however, the entomological inoculation rate, was ascertained to be the highest during 21:00 – 24:00 hours, the second quartile of the night [28]. Mean mosquito biting rates per person night were the lowest during November–April (winter months) and highest during May–October corresponding to wet season (Table 3). Parity rate was observed to be >60% supporting sporogony and has been widely incriminated by sporozoite in salivary glands infection rate ranging from anywhere 1.1%

-7.8% averaging ~3.0% [29]. Typically, good number of *Anopheles baimaii* mosquito adults were collected off-fences and thatched roofing in huts located in villages closer to forested areas after dusk (Figure 3).

Table 3. Seasonal biting rate and infectivity of *Anopheles baimaii* in a forest-fringe village of Dibrugarh district, Assam, NE India

Study period Month, year	Mean mosquito biting rate per person night	% Parous (parous /no. mosquitoes dissected)	% Anthropophilic index (mosquito +ve for human blood /no. dissected)	% Sporozoite infectivity rate (+ve for sporozoites / no. mosquitoes dissected)
June, 1999	80	69 (86/125)	67 (12/18)	1.1 (1/88)
July	45	93 (42/45)	100 (14/14)	6.7 (3/45)
August	38	41 (28/69)	97 (83/85)	0 (0/69)
September	27.5	71 (32/45)	84 (27/32)	5.8 (3/52)
October	27.5	92 (47/51)	95 (19/20)	7.8 (4/51)
November	1	50 (1/2)	100 (1/1)	0 (0/2)
December	0	-	-	-
January, 2000	0	-	-	-
February	0	-	-	-
March	1.5	67 (2/3)	100 (2/2)	0 (0/30)
April	1.7	20 (1/5)	75 (3/4)	0 (0/4)
May	43	61 (73/120)	75 (3/4)	2.3 (2/87)
Total	**6.31**	**61.5 (286/465)**	**91 (164/180)**	**3.2 (13/401)**

Source Reference [29]

Figure 3. A typical forest hut made of split-bamboo and thatched roofing in a forest-fringe village of Assam/ Arunachal Pradesh border in NE India (Courtesy, DR Bhattacharyya, Dibrugarh).

It is a hydrophilic species with population expanding in the wet-season associated with increased breeding resources and retracting back to mother foci in winter months. It is known to breed in jungle water transient pools/puddles shaded with dense foliage and animal footprints [30]. Populations of *An. baimaii* were assessed to be highly susceptible to residual insecticides (Table 4) [5, 31], yet this vector is considered invincible due to outdoor resting circumventing contact with sprayed surfaces and high anthropophagy [32].

Table 4. Insecticide susceptibility status of *Anopheles baimaii* in malaria endemic districts of North-East India

Location District, State [Reference No.]	Study period	Insecticide (diagnostic conc.)	No. of mosquitoes exposed	No. mosquitoes dead post 24 hours	Mortality (%)	Susceptibility status*
Dibrugarh, Assam [31]	October 1997	DDT (4%)	36	36	100	S
		Dieldrin (0.4%)	32	32	100	S
		Malathion (5%)	30	30	100	S
South Tripura, Tripura [5]	September 2012	DDT (4%)	10	10	100	S

*S = Susceptible

The habitat of *An. baimaii* is presently shrinking on account of depleting forest cover/broken reserve forest for increased anthropogenic activity for self-subsistence; its populations, however, even at lower threshold are considered sufficient for maintaining transmission of the deadly drug-resistant malaria [33].

Anopheles fluviatilis, a relay transmitter of malaria

An. fluviatilis s.l., is the predominant vector in hills and foothill areas of mainland India contributing ~15% of total reported cases in the country [34]. It is a medium sized mosquito having overlapping distribution records with *An. culicifacies* and can easily be identified given morphological identification keys [35]. It is a seasonal species reported to be prevalent in NE states in winter months beginning November until April and has been incriminated by detection of sporozoite infections in salivary glands (Table 5). Its density started building commencing November up until March and declined thereafter with the onset of warmer climates commencing April, and virtually disappeared thereafter. Sporozoite infections were registered in malaria-endemic areas of Assam as well as in hill ranges of Arunachal Pradesh that ranged between nearly 1–9 percent.

On account of its seasonal prevalence and infectivity, it is reckoned as relay vector of malaria supplementing transmission by *An. minimus* (the major vector in the NE region) in cooler months maintaining perennial transmission [3]. It is primarily a nocturnal biter and an endophilic species resting indoors in human dwellings in good numbers (inclusive of semi-gravid and gravid in nearly equal proportions) and held anthropophilic for having preference for human host. This species is known to share breeding habitats with *An. minimus* in seepage-water/slow moving streams/irrigation channels in foothill/plain areas (Figure 4). Aided by molecular taxonomic tools, populations of *An.*

Table 5. Seasonal abundance of *Anopheles fluviatilis* s.l. and infectivity in NE India

Month, Year	Location Block, District, State	Mosquito density per person hour	Vector Incrimination	
			No. individuals dissected	No. (%) gland positives
January, 1990	Dimoria, Kamrup, Assam	0.51	21	0
February	-do-	1.71	56	1 (1.78)
March	-do-	3.15	109	1 (0.92)
April	-do-	1.25	18	1 (5.56)
November	-do-	0.05	6	0
December	-do-	0.24	21	0
January, 1991	-do-	1.56	69	0
February	-do-	2.50	24	0
March	-do-	1.65	31	0
April	-do-	0.40	32	0
December, 1995	Yazali, Lower Subansiri, Arunachal Pradesh	2.07	54	5 (9.26)

fluviatilis in India have been characterized to be a species complex comprising of sibling-species S, T and U with varied eco-biological characteristics. All three sibling-species of this taxon can be identified by fixed chromosome inversion genotype as well as PCR based diagnostic assays [36, 37]. Among these, it is sibling-species 'S' which has been proven an efficient vector in range of its occurrence for its strong predilection for human host and records of its incrimination maintaining hyper-endemic malaria in post-monsoon months [38]. Given these molecular techniques, *An. fluviatilis* specific to Assam (earlier identified to be sibling-species 'U'), instead has now been characterised to be hyper-melanic seasonal variant of *An. minimus* s.s. prevalent in winter months [39]. This is further affirmed by homosequential banding patterns of chromosome arm-2 having similar inversion genotype in populations of both *An. minimus* and those morphologically identified as *An. fluviatilis*. Apparently, eco-biological characteristics of sibling-species 'S' of *An. fluviatilis* are remarkably similar to those of winter populations of *An. minimus* in Assam but does not merit synonymity with *An. fluviatilis* 'U' of lesser epidemiological significance as well as for appreciable genetic distances. Similarly, conspecificity of sibling-species 'S' of *An. fluviatilis* with that *An. harrisoni* (sibling-species 'C' of *An. minimus*) has been ruled out [40].

Both *An. minimus* s.l. and *An. fluviatilis* s.l. are indeed very closely related taxa belonging to Minimus subgroup, series Myzomyia of subgenus *Cellia* [41]. Apparently, *An. minimus* populations of Assam in winter months were earlier mis-identified as *An. fluviatilis* on account of melanism of wing and palpi characters that are taken diagnostic in morpho-taxonomy [35]. Paradoxically, populations of *An. minimus* inclusive of hyper-melanic variant (misidentified as *An. fluviatilis*) occurring in sympatry both in Assam and Arunachal Pradesh raises the possibility of existence of another taxon/haplotype similar to 'form V' reported in Uttarakhand, foothills of western Himalayas India having epidemiological significance [42].

Figure 4. Seepage /slow moving water streams are the preferred breeding habitat for both *Anopheles fluviatilis* and *An. minimus* in the foothills / plains of NE India. Mosquito breeding was recorded along grassy-banks sans sunlit areas.

Anopheles culicifacies, an emerging vector in degraded forests

An. culicifacies s.l. is a species complex comprising of 5 member species informally designated as A, B, C, D and E spread throughout India [43]. It is a dominant vector of rural malaria contributing 65% of reported cases in the country. Among these sibling species distributed in varied proportions across Indian landscape, species 'A' and 'E' are good vectors, 'B' is a poor vector, while 'C' and 'D' are of moderate significance. Historically, *An. culicifacies* is reported to occur in NE India but with dismally low population densities, therefore, considered of little significance [11]. With the advent of modern molecular tools, populations prevalent in the NE India were characterised to be sibling-species 'B' having no role in malaria transmission. However, in the last few decades there has been radical transformation of NE India on account of changing land use pattern associated with deforestation and population migration across borders resulting in altered ecology. In the present-day context, consequent to rollout of newer interventions particularly insecticide-treated nets for vector control, populations of major vector species namely *An. minimus* and *An. baimaii* are fast depleting and getting scarce. Instead, *An. culicifacies* is establishing its foothold and diversifying accessing niches vacated by *An. minimus* and *An. baimaii* [44]. Populations of *An. culicifacies* are now building and reported across malaria-ridden districts in good numbers with records of incrimination during focal disease outbreaks in localities formerly domain of latter two species (Table 6). Similar observations have been recorded in districts of central Assam reporting high build up in broken forests/ deforested pockets breeding in streams/irrigation channels [45]. Genetically, populations what formerly considered exclusively species 'B', are now believed to comprise of other sibling-species of the taxon having implications in vector control and are assessed to be multi-insecticide resistant posing renewed threat to control programme (Table 7).

There is a body of evidence that *An. culicifacies* is making in roads and fast emerging a vector of significance invading new territories in NE India replacing *An. minimus* and *An. baimaii* mandating continued surveillance. There is an urgent need to monitor its abundance to contain spread of multi-insecticide resistant populations and formulate appropriate strategies for effective control to avert spread of drug-resistant malaria.

Table 6. Abundance and infectivity records of *Anopheles culicifacies* s.l. in malaria-endemic districts of NE States of India

Location (Block, District, State)	Study period	No. mosquito adults collected	Mosquito density per person hour	Abdominal condition*				Parity**					Vector Incrimination	
				UF	FF	SG	G	No. mosquito dissected	NP	1P	2P	3P	No. mosquito dissected	No. (%) gland positive
Dimoria, Kamrup, Assam	Oct. 1990 – Sept. 1991	10	0.06	0	0	7	3	10	2	7	1	0	10	0
Manja, Karbi Anglong, Assam	May, 1991	13	0.28	-	-	-	-	-	-	-	-	-	13	0
Dimoria, Kamrup, Assam	Oct. 1991 – July 1992	31	0.60	-	-	-	-	27	9	15	3	0	31	0
Rangapara, Sonitpur, Assam	May – June 1992	69	1.06	4	8	38	19	35	10	18	7	0	68	1 (1.47)
Goreswar, Baksa, Assam	March 1995	73	1.4	-	-	-	-	-	-	-	-	-	73	0
Lakhipur, Goalpara, Assam	May 1995	102	2.49	-	-	-	-	91	58	32	1	-	102	0
Yazali, Lower Subansari, Arunachal Pradesh	Dec., 1995	5	0.18	-	-	-	-	-	-	-	-	-	5	0

*UF= Unfed, FF= Fully fed, SG= Semi-gravid, G = Gravid **P = Parity (number of oviposition cycles), NP = non-parous, 1P = Uniparous, (-) denotes data not collected

Table 7. Insecticide susceptibility status of *Anopheles culicifacies* s.l. in malaria-endemic areas of Assam, NE India

Location (Block, District)	Study period	Insecticide (Diagnostic conc.)	No. mosquitoes exposed	No. mosquitoes dead post 24 hrs exposure	Mortality (%)	Status*
Paneery, (Udalguri)	May 2011	DDT (4%)	35	14	40	R
		Malathion (5%)	20	18	90	VR
Sidli, (Chirang)	September 2011	DDT (4%)	8	2	25	R
		Deltamethrin (0.05%)	7	3	30	R

*R = Resistant (<90% mortality), VR = Verifiable resistance (90 – 97% mortality)

Malaria vectors of secondary importance

Anopheles philippinensis/Anopheles nivipes

An. philippinensis is a common mosquito species recorded to be prevalent across north-eastern states, populations of which are now known to comprise of both *An. philippinensis* and *An. nivipes*, the two very closely and often morphologically indistinguishable species, in varying proportions [46]. Both are closely related species of *An. annularis* group and often misidentified owing to subtle morphological differences [35]. In earlier records, populations of these species were invariably recorded as *An. philippinensis* specific to NE India. But these two species can now be identified based on polytene chromosome banding pattern as well as allele specific polymerase chain reaction (ASPCR) method [47]. Among populations screened across NE states collected from varied ecotypes [46, 48], it was observed that *An. nivipes* was relatively more widespread and predominant species in Assam, Manipur, Nagaland, Meghalaya and Tripura states, whereas *An. philippinensis* was most abundant in hill states of Mizoram and Arunachal Pradesh (Figure 5).

An. nivipes mosquitoes, however, were recorded to be abundant in forested villages of both plain and hilly tracks amidst paddy-fields (<200 msl) and the least in deforested areas. Instead, *An. philippinensis* was relatively a species of the hills (400 - 600 msl), but much less of plains; nevertheless, in the upper hill ranges both species were recorded in much less density. These species were recorded breeding profusely in paddy fields (the preferred breeding habitat), most abundant in the wet season (April–September) and largely zoophagic as evidenced by host bloodmeal analysis (99% cattle blood, 343/346). Both species are exophilic and can be collected in good numbers feeding on cattle after dusk and are assessed to be highly susceptible to malathion and pyrethroids although data on susceptibility status of individual species still not available and requires confirmation [49]. However, there are records of collections off human host for data based on dusk-to-dawn human-landing catch; seasonal mosquito biting rate, however, varied from 0.50–4.50 per person night with peak during August–October corresponding to seasonal abundance [19].

An. philippinensis/nivipes were implicated in disease transmission through detection of sporadic sporozoite infection in salivary glands [50] as well as by ELISA through detection of circumsporozoite protein (CSP) antigen of both *P. falciparum* and *P. vivax* (1.68%, 28/1670) [51], and nested PCR (0.5%, 2/411) for parasite positivity [52]. Thus, these species

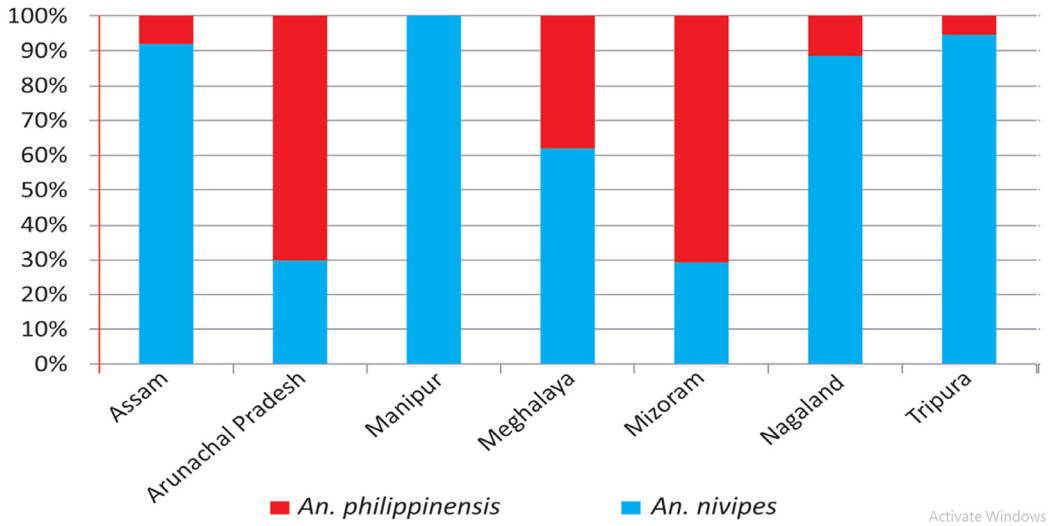

Figure 5. Relative abundance of *Anopheles nivipes* and *Anopheles philippinensis* mosquito species in north-eastern states of India. Source Reference [46]

hold the potential of resuming transmission in the wake of disappearing major vector species for which continuous entomological surveillance is mandated to contain residual transmission in the context of malaria elimination.

Anopheles varuna

In NE India, *An. varuna* is a close companion of *An. minimus* and invariably found resting indoors in human dwellings constituting fair proportion of the mosquito fauna [18,19,53]. Both species belong to *An. minimus* group of mosquitoes and are very similar, often misidentified, for subtle morphological differences [35,54]. Yet these two species differ in bionomical characteristics for their relative abundance in different biotopes as well as disease transmission relationships. Unlike *An. minimus*, populations of *An. varuna* were also recorded in cattle-sheds, whereas relative abundance in human dwellings was comparatively much less; besides, there exists no record of infectivity in *An. varuna* specific to NE India except rare occurrence of CSP antigen for *P. vivax* by ELISA [12]. In contrast to *An. minimus* breeding exclusively in slow-flowing seepage water streams, larval breeding of *An. varuna* were recorded in variety of habitats including ponds, roadside ditches, pits and paddy fields [55]. Comparatively, mosquito biting rate was significantly lower compared to *An. minimus* and varied from 0.25 – 5.25 per person night across seasons [19]. Perhaps due to its low epidemiological significance, its biology is poorly understood something which needs to be prioritized to rule out its role in disease transmission post-elimination.

Anopheles aconitus

An. aconitus is a member species of the *An. minimus* group and have been reported resting

both in human dwellings indoors and cattle-sheds, however, its relative abundance was much less (<1 per person hour) compared to *An. minimus* and *An. varuna* [53]. It is largely zoophagic and exophilic found resting outdoors, yet good number of mosquitoes were recorded in overnight human-landing catches during June – September and mean mosquito biting rate was 3.47 per person night [19]. It is recorded breeding in ponds, streams, pits and occasionally in paddy-fields [55]. There are records of occasional infections in Assam along with parasite positivity based on CSP antigen assays (3.95%) [12].Based on the available information, there exists possibility of supplementing transmission by this species in conjunction with other major operating species in the NE India. However, more data are warranted to delineate its distribution and its role helping formulate comprehensive intervention strategy.

Anopheles jeyporiensis

Besides major vector species in NE India, *An. jeyporiensis* should be the prime suspect in supplementing transmission for its shared bionomical characteristics with *An. minimus* but has largely been ignored for lack of data on infectivity. *An. jeyporiensis* has been reported resting both indoors and outdoors but in lesser proportions compared to *An. minimus* [53]. It was also recorded breeding in slow-flowing seepage water streams as well as in fallow rice fields [55]. Akin to *An. minimus*, it is equally recorded foraging human bloodmeal in overnight catches but mosquito biting rate per person night varied between malaria endemic areas, viz., it was 0.24 in Assam [19] vis-à-vis 2.50 per person night (indoors) in Tripura reporting focal disease outbreaks [5]. There are no records of its incrimination except those of CSP antigen capture assay reporting minimum prevalence infection as high as 6.25% in Assam [12]. Given these attributes, it is likely that *An. jeyporiensis* may supplement transmission in areas of its distribution, but additional data are warranted to estimate its vectoral capacity helping devise appropriate policy for containment.

Anopheles maculatus

An. maculatus is a species group comprising of nine closely related species of which six namely *An. dravidicus*, *An. maculatus s.s.*, *An. pseudowillmori*, *An. rampae*, *An. sawadwongporni*, and *An. willmori* are recorded to occur in NE India in varying proportions [56]. Among these, *An. pseudowillmori* (59.5%) and *An. maculatus* s.s. (32%) were identified to be most abundant and rest member species occurred in insignificant numbers. Member species of the Maculatus group can be characterized unequivocally by number of techniques including karyotypic studies and PCR based molecular assays [57]. In earlier literature records, all these species were reported as *An. maculatus* except reference of two varietal forms [58]. It is largely a species of hills and foothills than plain valleys, but relative abundance varied in different ecotypes (Table 8).

It is predominantly a zoophagic and exophilic species and considered of low epidemiological significance for lack of incrimination records. However, *An. pseudowillmori* has been reckoned a potential vector in Bhutan sharing vast border with Assam/Arunachal Pradesh [60]. There exists possibility of contributing malaria cases by this species in NE India as well

Table 8. Relative abundance of *Anopheles maculatus* s.l. in different ecotypes of malaria-endemic areas of NE region

Study location (district, state)	Ecotype	Study period	Mosquito density per person hour		Mosquito-biting rate per person night		No. mosquitoes per CDC light trap night	
			Day-resting	Cattle-biting	Out-doors	In-doors	Out-doors	In-doors
Dimoria block (Kamrup, Assam)	Plain valleys	Oct.1988-Sept.1990	0.06	0.24	-	0.18	-	0.09
Silachari (South Tripura, Tripura)	Indo-Bangladesh border	June-Sept. 2012	0	0.73	1.33	0.50	5	3
Jairampur (Changlang, Arunachal Pradesh)	Foothills of Indo-Myanmar border	Feb-Sept., 1990	-	2.44	0.67	0.55	2.52	-

Source Reference [59]

due to high anthropophagy as evidenced by host-blood meal analysis of *An. pseudowillmori* (50%, 17/34) and *An. willmori* (65%, 13/20) that are widely prevalent in the region [56]. *An. maculatus* mosquitoes were recorded breeding in variety of habitats including ponds, seepage water streams, pits and paddy-fields [55]. There is paucity of information on insecticide susceptibility status specific to NE India helping formulate control strategy. Member species of this group are believed to be fast evolving given the diverse breeding habitats and ecology role of which should be considered in context of disappearing malaria in NE region and emergence of zoonotic malaria making inroads in Southeast Asia.

Anopheles annularis

An. annularisis a species group comprising of 5 currently recognized species of which 4 are reported to occur in India,viz., *An. annularis*, *An. nivipes*, *An. pallidus* and *An. philippinensis* which can be characterized unequivocally by PCR-RFLP method [61, 62]. *An. annularis* species has been characterised to be comprising of two forms provisionally designated as 'A' and 'B', of which 'A' is known to be prevalent in NE India. These two cryptic species can be characterized based on polytene chromosome karyotype as well as PCR based rDNA restriction fragment length polymorphism [63,64]. *An. annularis* is predominantly a zoophilic species easily collected in cattle-sheds, however, a proportion of its populations were also found resting in human dwellings indoors [53]. *An. annularis* has been incriminated in Odisha [65] and West Bengal [66]. It is though not considered vector of any importance in the NE India, yet historically it was suspected to be playing some role in malaria transmission in Goalpara district of Assam [58]. In spite of indication of predilection for human host based on mosquito biting rate (1.15 per person night) as well as reporting of 5.8% minimum infection rate in ELISA-CSP antigen capture assay, not a single individual of this species dissected for salivary glands was found sporozoite positive [12,51]. *An. annularis* species is reported to share breeding with several other *Anopheles* species in ponds, ditches, streams and paddy fields [55], and its populations are reportedly resistant to DDT, but susceptible to malathion and pyrethroids [67].

Conclusions

High receptivity to malaria in NE India can be largely attributed to multiple vector species, ambient environment, and ecology conducive for mosquito proliferation and longevity. Among six dominant mosquito vectors of malaria in India [68], two species namely *An. minimus* (perennial species) and *An. baimaii* (monsoon species) native to the NE region are by far the most efficient for high anthropophagy and sporozoite infectivity; together these are responsible for focal outbreaks taking high toll on human lives. Both these species are susceptible to residual insecticides and given the rollout of LLIN based interventions in force, populations of these species are fast depleting. Instead, *An. culicifacies*, populations of which are multi-insecticide resistant, is occupying niches and diversifying with proven records of its infectivity. *An. fluviatilis* (winter species) is just an efficient vector, however, true abundance of this species requires further verification across NE states in view of characterization of its populations as seasonal variant of *An. minimus*. There are indications of *An. philippinensis/nivipes* (the most predominant species) supplementing transmission based on sporadic infectivity but largely these are zoophagic. Among other probable vector species, *An. jeyporiensis* is the one that can be suspected to be playing some role in transmission for having bio-ecological characteristics akin to *An. minimus*, but poorly explored. The role of *An. varuna*, *An. aconitus*, *An. maculatus* and *An. annualris* would only be marginal, if any, that too subject to build up of high densities. Vector control is an integral component of malaria control strategies for which building capacities at zonal and national level are critical for entomological surveillance enabling interventions in place and time; an expertise that is fast waning [69-71]. Universal coverage of interventions should be the cornerstone to ward off the dreaded vector species; laxity and complacency can prove to be counterproductive and costly [72,73].

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Section III

Epidemiology of Malaria Transmission

5

Malaria transmission in Assam, an historic account, and current perspectives

Introduction

Assam is the major state (24°44′- 27°45′N latitude; 89°41′-96°02′E longitude) constituting nearly 70% of total population of North-East India, and by far the most developed for its industrial productivity, infrastructure, educational institutions, and access to healthcare services. Historically, malaria has been the major public health concern in the state and subject for investigations for its containment beginning with naturalistic interventions (pre-independence) to current tools including insecticide-treated netting materials for vector control and newer antimalarials for treatment of drug-resistant malaria [1-3]. It is co-endemic for both *Plasmodium falciparum* and *P.vivax* malaria with reports of devastating disease outbreaks taking heavy toll on morbidity and human lives [4,5]. Transmission remained uninterrupted in large tracts of land for decades affecting most districts due to lack of information on disease distribution and determinants, inadequate surveillance (that can best be described as fragmented) and requisite interventions in place and time [6]. Communities little aware of disease prevention and control remained at risk for long perpetuating vicious cycle of malaria and poverty. In the past few decades, research efforts have generated a great deal of information on disease vectors and transmission dynamics, and evaluation of community-based newer intervention tools to help check spread of malaria [7]. Given the present-day knowledge on vector bionomics, transmission dynamics and rollout of intervention tools, Assam has made huge strides with expanding infrastructure of rail and road communication networks and healthcare outreach services. To maintain productivity, containing infectious diseases remained the utmost priority for harmonious growth across strata. Among these, malaria has been in the forefront requiring prioritization by programme and policy managers for greater allocation of resources and continued political support for strengthening healthcare services. In this chapter, the magnitude of malaria problem and changing transmission profile over space and time, is presented enabling spearheading targeted interventions to end transmission for good in keeping with global agenda for malaria-free world by 2030.

Topography and climate

Assam is the most populous (estimated to be 35 million in 2020) and second largest state of North-East India sharing borders with all other sister states (Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland and Tripura) and international borders with Bhutan to the north, Myanmar to the east and Bangladesh to the south (Figure 1). The landscape is marked by major river systems namely the mighty Brahmaputra running across the entire length of the state from east to west (Brahmaputra valley), and the Barak River in south Assam (Surma valley); both are the lifeline supporting livelihood of

masses. Most people live in rural villages; town areas are few and far except capital city of Guwahati (the gateway to northeast) having dense urban agglomeration, however, it is not categorized under Urban Malaria Scheme of the National Vector Borne Disease Control Programme.

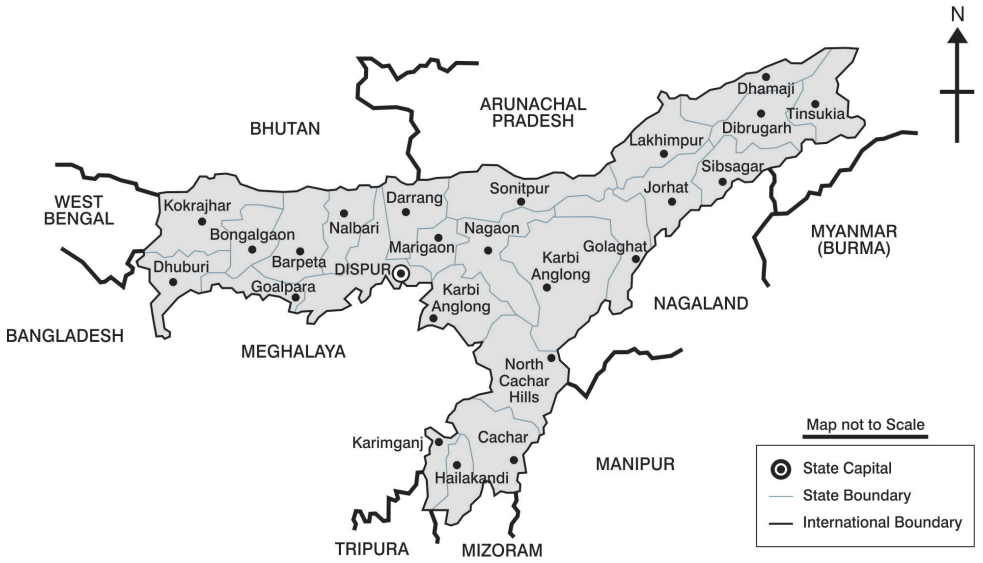

Figure 1. Political map of Assam showing interstate border with its sister states of Arunachal Pradesh, Nagaland, Manipur, Mizoram, Tripura and Meghalaya, and geographical proximity to adjoining countries of Bhutan, Myanmar and Bangladesh. Additional districts were created post 2004.

Ecological conditions are congenial for mosquito proliferation throughout the state for most part of the year. In the valleys, temperatures are warmer and are highly receptive for malaria transmission associated with high humidity. Typically, summers are hot and humid with maximum mean monthly temperatures ranging from 22^0 to 32^0C, and winters are mild with minimum monthly temperatures ranging from 9^0– 26.5^0C. Invariably, the lowest temperatures (9.0^0C – 9.5^0C) are recorded in winter months of December and January (Table 1). Throughout Assam, it rains heavily spanning six months of the year during April – September and total precipitation varied anywhere from 1500 – 2000 mm spread over 100-140 days per annum (Table 2). The state can be conveniently divided into three main regions namely, Upper, Central and Lower Assam. Temperatures get cooler in relation to elevation from sea level and rainfall tends to be higher (2000 – 3000 mm) in Central and Upper Assam comparative to districts of lower Assam in the valley.

Most districts are plain except two hill districts namely Karbi-Anglong and Dima Hasao (formerly North Cachar hills) with elevation up to 3000 feet above mean sea level (msl). These are also the districts dominated by indigenous tribal populations heavily infested with malaria. Nearly 40% land of the state is under forest cover rich in wildlife, but forest reserve that varies between districts, is depleting at expense of population explosion, migration across borders and industrial expansion resulting in ecological shift affecting fauna and flora. Paddy cultivation is the major occupation, while others included handlooms (Assamese silk) and forest produce. Tea is the major cash crop along with coal, oil and natural gas exploration, rubber plantation, jute and timber, adding to the state revenue.

Table 1. Meteorological data of Sonitpur district, Central Assam, North-East India*

Month (1991)	Mean Temperatures ^{0}C		Rainfall (mms)
	Maximum	Minimum	
January	22.5	9.0	17.4
February	25.5	13.5	34.6
March	29.0	17.5	52.8
April	28.0	19.5	113.6
May	27.0	22.0	473.2
June	30.0	25.5	574.2
July	32.5	26.5	329.4
August	31.5	26.0	383.4
September	30.5	25.0	479.2
October	29.5	22.0	244.2
November	27.0	14.0	13.2
December	22.5	9.5	23.0

*Source: Tarajulie Tea Estate, Assam (India)

Table 2. Meteorological data of Kamrup district, Lower Assam (1991 – 2003), North-East India*

Year	Rainfall data		Mean Temperatures ^{0}C	
	Total rainfall (mm)	No. of rainy days	Maximum	Minimum
1991	2015	119	28.9	19.6
1992	1845	103	28.9	19.2
1993	2120	117	28.8	19.6
1994	1546	114	29.6	19.3
1995	2169	121	29.5	19.7
1996	1559	110	29.9	19.8
1997	1568	117	29.2	19.2
1998	1727	127	29.6	19.8
1999	1793	110	30.5	20.3
2000	1804	99	29.2	19.7
2001	1755	118	29.8	20.0
2002	1695	127	29.3	20.1
2003	2071	138	29.0	20.1

*India Meteorological Department, Meteorological Centre, Guwahati, Assam (India)

Malaria-attributable morbidity and mortality

Malaria is endemic in Assam and continued to be deterrent affecting equitable socio-economic development of the state. Despite interventions in force encompassing residual spraying against vectors and disease surveillance, transmission continued to be perennial and persistent throughout the valley [8]. All districts were reporting malaria-attributable morbidity; transmission intensities, however, varied between districts [9].

Among commonly known four human plasmodial species, *Plasmodium falciparum* and *P. vivax* occurred in abundance across the state. However, of the other known plasmodial species, *P. malariae* were reported in good numbers in districts of upper Assam in pre-independence India [10], but subsequently this species almost disappeared except few sporadic cases that continued to resurface in the region [11,12]. *P. ovale*, instead has not been reported in the northeast India except a lone case in Assam, but its transmission could not be substantiated [13].

Based on disease surveillance both inclusive, i.e., active case detection (door-to-door fever surveys) and that of passive case detection (self-reporting with fever in the malaria clinic), malaria positive cases were recorded each year with flare up few years apart largely attributed to focal disease outbreaks associated with increased morbidity (Table 3). Based on microscopic examination of blood-smears, *P. falciparum* was predominant proportions of which ranged anywhere between 55 – 77 percent during 1991-2020; the remaining were *P. vivax* cases. During these years, up until 2010, annual parasite incidence (API) remained >2 (that means two positive cases per thousand population), thereafter steady decline was observed from 1.48 in 2011 to 0.01 in 2020. Disease surveillance (annual blood examination rate), however fell short of the target 10% of population in good number of years amounting in inadequate surveillance attributed to operational constraints, viz., incessant rains and floods restricting access, insurgency, complacency and lack of supervision etc.

Malaria-attributable deaths were recorded each year but remained well below 100 except during outbreak years notably in 1995, 1999, 2001, 2005, 2006 and 2007. Each death case was attributed due to *P. falciparum* infection confirmed positive based on peripheral blood-smear examination. Nevertheless, 2011 onwards a steady fall in malaria cases and deaths was observed owing to strengthening interventions both against parasite and disease vector species prioritizing the high-risk areas. Malaria cases were consistently recorded in all age groups and gender inclusive of infants ($\leq$1 years); concentration of cases, however, was significantly higher in (5 – 15) and >15 years age groups (Table 4). Likewise, malaria-attributable death cases were also observed in all age groups of both sexes; however, death toll had risen significantly during outbreak years in commensurate with rising *P. falciparum* cases (Table 5).

Seasonal distribution of cases and transmission dynamics

Malaria cases were recorded in all months of the year [4,8]. Both *P. falciparum* and *P. vivax* occurred in abundance, however the former remained the predominant infection. Perennial active transmission of this pathogen was reaffirmed by parasite positivity in infants(<1 years of age) along with gametocytes (Table 6). Gametocyte carriage, however, was greater in 5-15 years age group more so during winter months (October – December). Seasonal peak was observed in commensurate with onset of pre-monsoon showers in April with peak during May - September/October corresponding to wet season (Figure 2).

Typically, the seasonal peak was largely held due to high rise in *P. falciparum* cases. For remainder of the year, cases were observed but much less in number. The transmission pattern was consistent, but intensities decreased each passing year commencing 2010 in relation to interventions in force. With the rollout of available intervention tools and strengthening healthcare services, Assam is presently fast accelerating towards malaria elimination in the foreseeable future (Figure 3).

Table 3. Malaria-attributable morbidity and mortality data based on disease surveillance in Assam (1991 – 2020)*

Year	No. of blood-smears examined for malaria parasite	Total smears +ve for malaria	No. *P. falciparum* cases (% of total smear +ve cases)	Annual blood examination rate (% of population checked for malaria)	Annual parasite incidence (No. of cases/1000 population)	No. of deaths
1991	2412379	107572	72994 (68)	10.25	4.57	36
1992	2343332	95168	62248 (65)	9.82	3.98	20
1993	2684250	118403	78504 (66)	11.09	4.89	48
1994	2745256	160538	105495 (66)	11.21	6.55	69
1995	3254120	230702	145153 (63)	13.13	9.31	202
1996	2987382	176622	107742 (62)	12.85	7.13	58
1997	2688224	123650	76511 (62)	10.54	4.85	27
1998	2441084	94645	54769 (58)	9.34	3.62	34
1999	2872859	131048	83064 (63)	10.78	4.91	111
2000	2215375	84915	52116 (61)	8.23	3.15	43
2001	2432620	95142	58951 (62)	8.90	3.48	122
2002	2325105	89601	55825 (63)	8.32	3.21	72
2003	2133820	76570	48647 (71)	7.66	2.74	53
2004	1853560	58134	41400 (67)	6.45	2.02	52
2005	2050261	67885	45453 (65)	7.06	3.34	113
2006	2743092	126178	82546 (69)	9.35	4.30	304
2007	2399836	94853	65542 (69)	8.09	3.19	152
2008	2687756	83939	58124 (73)	8.62	2.74	86
2009	3021915	91413	66557 (72)	9.66	2.92	63
2010	4309267	66716	48330 (73)	13.75	2.13	36
2011	4130216	47397	34807 (69)	12.89	1.48	45
2012	3973341	29999	20579 (77)	12.24	0.92	13
2013	3895330	19542	14969 (77)	11.83	0.59	7
2014	3684068	14540	11210 (77)	11.08	0.44	11
2015	3485405	15557	11675 (75)	10.37	0.46	4
2016	3032997	7826	5686 (73)	8.94	0.23	6
2017	2669423	5281	3494 (66)	7.74	0.15	0
2018	2364621	3816	2859 (75)	6.75	0.11	2
2019	4352477	1459	872 (60)	12.22	0.04	4
2020	3246745	484	266 (55)	9.01	0.01	2

*Source: State Health Directorate of Assam

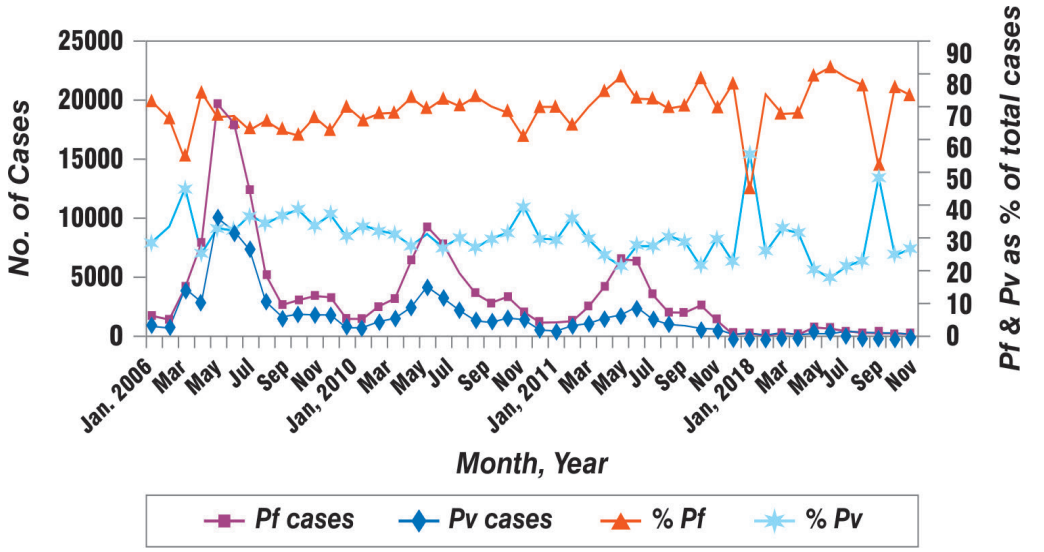

Figure 2. Transmission of *Plasmodium falciparum* and *Plasmodium vivax* malaria for data based in Assam. During 2006 *P. falciparum* cases were treated with sulfadoxine-pyrimethamine (SP), and *P. vivax* with chloroquine + primaquine for 5 days schedule @ 0.25mg/kg/day. Beginning of 2007, SP was replaced with artesunate + SP (ASP) for *P. falciparum* cases, while for *P. vivax* 5-day course of primaquine was replaced with 14-day treatment @ 0.25 mg/kg/day for radical cure. In 2013, drug-policy was upgraded replacing ASP with AL (artemether + lumefantrine) for treatment of *P. falciparum* specific to North-East India (data source: State Vector Borne Disease Control Programme of Assam). Pf and Pv denotes *Plasmodium falciparum* and *Plasmodium vivax* cases respectively; % Pf and %Pv are derived by division of number of cases of respective species by total positive malaria cases.

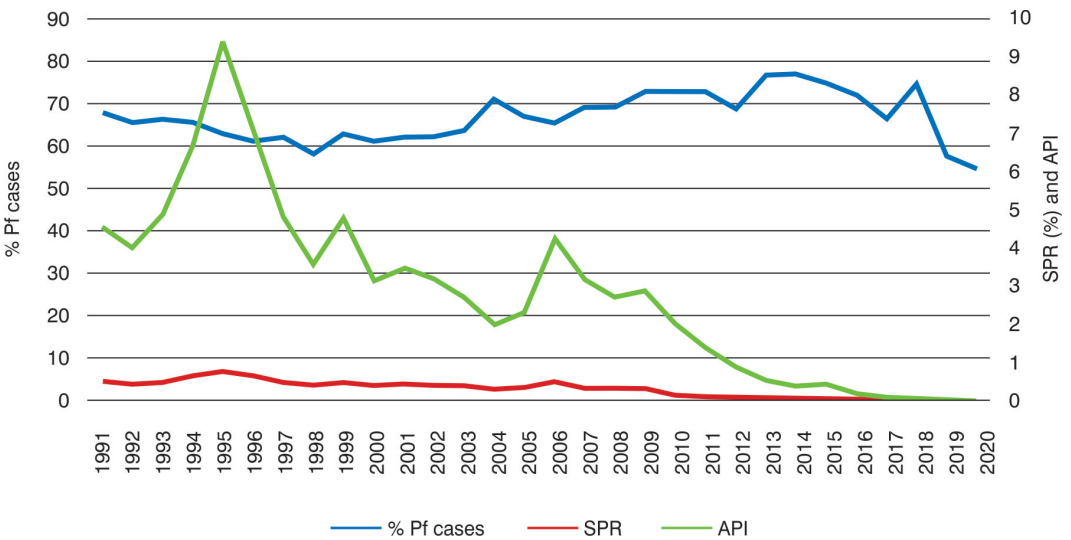

Figure 3. Malaria transmission in Assam (1991-2020). SPR denotes smear positivity rate of blood-smears examined for malaria parasite. Pf = *Plasmodium falciparum*. %Pf relates to per cent of *P. falciparum* cases of total positive cases for any malaria parasite. API represent number of malaria positive cases per 1,000 population year. Data source: National Vector Borne Disease Control Programme, India

Table 4. Distribution of malaria cases by age and gender in high-risk villages (population 29,537) of the Sonapur Primary Health Centre, Kamrup district, Assam, North-East India*

Age group (years)	Gender	No. of fever cases	Total blood-smears +ve for malaria	No. of *P. falciparum* cases (% of total +ve cases)	Parasite rate (%)
<1	Male	116	26	20 (77)	22.4
	Female	110	26	19 (73)	23.6
1 - <5	Male	879	246	196 (80)	27.9
	Female	889	277	213 (77)	31.2
5 - 15	Male	1863	609	518 (85)	32.7
	Female	1874	556	473 (85)	29.7
>15	Male	4193	823	727 (88)	19.6
	Female	3553	706	580 (82)	19.9
Any		**13477**	**3269**	**2746 (84)**	**24.3**

*Data based on active fever surveillance during January – December 1989

Table 5. Distribution of malaria-attributable deaths across age groups and gender in outbreak years of 1995 and 1999 in Assam, North-East India

Age group (years)	No. of deaths in 1995		No. of deaths in 1999	
	Female	Male	Female	Male
0 - < 5	9	7	8	8
5 – < 10	16	14	3	8
10 - < 15	13	12	6	7
15 - < 45	39	64	20	39
45 – 59	7	15	0	7
> 59	0	6	1	4
Any	84	118	38	73

Source Reference [4]

High-risk districts and prioritizing interventions

Even though all districts are endemic for both *P. falciparum* and *P. vivax*, relative abundance varied among districts (Table 7). Over the past decade, several districts formerly considered high-risk [14], have moved to low-risk for having contained *P. falciparum* to large extent. Based on data for 2019, *P. falciparum* remained the predominant infection in hill districts of N.C. Hills (Dima Hasao) and Karbi Anglong, and those of Goalpara, Kamrup (Rural), Cachar and Udalguri constituting 69% - 94% of total reported cases, while in Chirang, Kokrajhar, Dhubri and Karimganj, both parasite species occurred in nearly equal proportions (a radical shift from previous years for reduced risk for contracting *P. falciparum* malaria). In the remaining districts, *P. vivax* cases far exceeded those of *P. falciparum*.

Given the reported cases, districts of Udalguri and Kokrajhar (reporting 400 - 450 cases) are seen high-risk, whereas Karbi Anglong and Chirang (reporting 80 - 100

Table 6. Monthly distribution of *Plasmodium falciparum* cases by age based on active fever surveillance in high-risk villages (population, 22000) of the Sonapur Primary Health Centre, Kamrup district, Assam, North-East India

Month (1991 - 1992)	<1 year		(1 – < 5) years		(5 – 15) years		>15 years	
	No. of blood - smears examined	+ve for *Plasmodium falciparum* (g)*	No. of blood - smears examined	+ve for *Plasmodium falciparum* (g)*	No. of blood - smears examined	+ve for *Plasmodium falciparum* (g)*	No. of blood - smears examined	+ve for *Plasmodium falciparum* (g)*
Oct, 1991	20	4 (0)	113	35 (1)	282	80 (11)	428	85 (2)
Nov	14	5 (0)	91	22 (1)	276	66 (4)	421	85 (3)
Dec	10	1 (0)	67	18 (1)	237	62 (3)	341	48 (2)
Jan, 1992	12	1 (0)	94	19 (2)	140	32 (5)	297	30 (0)
Feb	17	5 (1)	82	25 (1)	125	25 (0)	337	20 (1)
Mar	8	0 (0)	69	6 (0)	172	13 (1)	282	23 (1)
Apr	9	2 (0)	84	18 (0)	145	34 (0)	254	47 (0)
May	13	1 (0)	111	20 (0)	272	58 (0)	444	73 (1)
Jun	16	2(0)	127	20 (2)	310	70 (4)	474	79 (1)
Jul	20	8 (1)	122	53 (0)	402	183 (3)	566	224 (1)
Aug	16	5 (1)	126	49 (3)	326	105 (7)	466	122 (4)
Sep	15	4 (0)	75	26 (2)	255	78 (7)	363	60 (1)

*(g): smears positives for *Plasmodium falciparum* with gametocytes

cases) posed medium risk (Figure 4). These are also the tribal dominated territories requiring enforced interventions to mitigate the risk of impending disease outbreaks and spread of drug-resistant malaria. All other districts were categorized low-risk reporting <80 cases/year.

Table 7. Distribution of malaria cases in districts of Assam for data based on 2019*

District	Population	No. blood-smears examined for malarial parasite (% of population checked)	No. blood-smears +ve for malaria parasite		% of malaria cases +ve for *Plasmodium falciparum*	Annual parasite incidence (No. of cases/ population x 1000)	No. of death cases
			Plasmodium vivax	*Plasmodium falciparum*			
Barpeta	1688818	178410 (11)	11	1	8	0.007	1
Bongaigaon	776164	132394 (17)	10	1	9	0.014	0
Baksa	1015030	158022 (16)	7	3	30	0.009	0
Cachar	1902074	166292 (9)	2	31	94	0.017	0
Chirang	542286	78311 (14)	42	46	52	0.162	0
Darrang	1033362	139171 (13)	7	3	30	0.009	0
Dhemaji	753149	118651 (16)	4	1	20	0.006	0
Dhubri	2082030	192511 (9)	12	12	50	0.011	0
Dibrugarh	1408826	153694 (11)	6	1	14	0.005	0
Goalpara	1130310	239596 (21)	11	47	81	0.051	0
Golaghat	1113477	158225 (14)	4	0	0	0.003	0
Hailakandi	777589	114806 (15)	4	0	0	0.005	0
Jorhat	947106	117431 (12)	5	2	29	0.007	0
Kamrup (R)	1743161	322593 (18)	1	11	92	0.007	0
Kamrup (M)	1163149	129331 (11)	5	3	38	0.007	0
Karbi-Anglong	1143767	147813 (13)	10	85	89	0.083	0
Karimganj	1421858	142945 (10)	27	29	52	0.039	2
Kokrajhar	991959	136953 (14)	219	228	51	0.450	1
Lakhimpur	1245810	148834 (12)	0	0	0	0	0
Morigaon	1031503	165342 (16)	9	5	36	0.013	0
Nagaon	3102606	362600 (12)	22	18	45	0.012	0
Nalbari	873477	119164 (14)	3	1	25	0.004	0
N.C. Hills	257823	55734 (22)	7	57	89	0.248	0
Sibsagar	1180642	141334 (12)	2	1	33	0.002	0
Sonitpur	2104249	296813 (14)	16	8	33	0.011	0
Tinsukia	1318617	132411 (10)	4	0	0	0.003	0
Udalguri	913862	103096 (11)	128	287	69	0.454	0
Total	33662704	4352477 (13)	587	872	60	0.043	4

*Source: State Health Directorate of Assam

Conclusions, and the way forward

The advent of newer intervention tools namely the long-lasting insecticidal nets (LLINs) for vector control and rollout of artemisinin-based combination therapies (ACTs) for treatment of malaria have ushered a new era of hope in malaria control operations [15]. The increasing coverage prioritizing high-risk populations have indeed made tangible impact in malaria transmission almost reaching pre-elimination stage in 2019 reporting 98% reduction compared to 2010 levels (Table 3). Number of malaria-attributable death cases also declined by 89% in commensurate with parallel decrease in *P. falciparum* cases in the corresponding period. However, as per WHO estimates [16], the reported cases and deaths may only be miniscule of the actual incidence on account of inadequate disease surveillance, poor-reporting, and cases not included those captured by private and public sector establishments. The disease burden due to *P. vivax* malaria (presently grossly underestimated) is indeed enormous and likely to emerge major challenge, the one that would be difficult to eliminate due to its relapsing characteristics years apart even beyond post-elimination [17]. Nonetheless, the reported case incidences revealed disease transmission trends that are surely and steady deaccelerating presenting window of opportunity aiming universal coverage to end transmission.

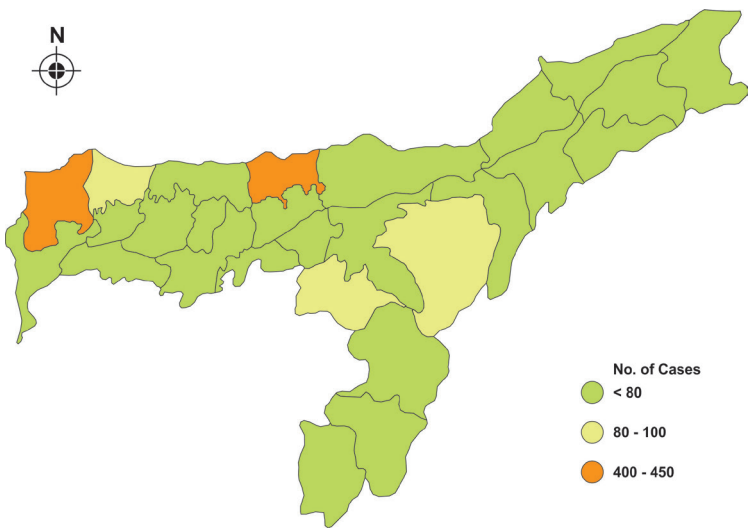

Figure 4. Stratification of districts of Assam by number of malaria cases for data based on 2019. Districts reporting 400 – 450 cases are high-risk, whereas districts reporting 80 – 100 and those < 80 cases are considered medium and low-risk, respectively. Data source: State Health Directorate of Assam

The road to malaria elimination seems achievable but miles to go before the finish line [18]. Euphoria may not last very long; malaria has history of return with vengeance [19]. Complacency at this stage of achievement at any level of control operations would be catastrophic. A host of challenges lie ahead which need to be addressed to defeat the killer parasite enumerated as follows: (i) first and foremost would be to continue monitoring therapeutic efficacy of antimalarials and upgrading drug-policy to thwart spread of drug-resistant varieties, (ii) ascertain the magnitude and distribution of asymptomatic malaria and formulate policy to mitigate the hidden foe to disrupt community transmission, (iii) strengthen capacity for entomological surveillance to monitor vector population density and emerging ecological succession, and monitoring insecticide susceptibility status to

ward off the disease carriers, (iv) to ensure universal coverage of interventions (inclusive of both LLINs and ACTs) which presently is far from adequate to protect populations at any risk, (v) to seek continued political commitment for sustained supply of logistics to avert disease outbreak and spread of malaria, (vi) above all to address cross-border malaria by strengthening health systems for data sharing and coordinated interventions (the marginalized population groups dwelling in the forest-fringe/inter-border areas are prone to disease outbreaks that carry brunt of the disease burden). The paradigm of cross-border malaria would gain eminence post-elimination to prevent entry in malaria-free territories [20].

Taken together, all these measures would help continued success paving the way to end malaria transmission in the state. Strengthening interventions in Assam (the main corridor of economic activities in the northeast), should be the priority for greater allocation of resources for universal coverage; investment in this state would yield more dividend to contain spread of drug-resistant malaria in the peninsular India and beyond. Equity in healthcare access and affordable treatment holds the key to keep malaria at bay. Defeating malaria in Assam (formerly considered intractable) would certainly be a landmark achievement in public health and prove to be the forerunner for achieving coveted goal of malaria elimination not only in the country but also in the neighbouring countries of Bhutan and Myanmar fast accelerating towards malaria elimination [21,22].

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6

Malaria clinic (passive case detection): a strong hold in malaria research

Introduction

In the national malaria control programme, besides vector containment measures, case detection and treatment are the major stay encompassing both active surveillance as well as passive case detection (malaria clinic), a built-in component in the primary healthcare services (PHC) at the block level [1]. Each block PHC is well equipped with microscopic facility for screening malaria parasite and providing access to the treatment in the catchment population. In this context, while early case detection and prompt treatment (EDPT) has been the key-intervention in reducing morbidity and preventing deaths, malaria clinic instead has been beneficial innumerable ways helping understand local disease epidemiology which can prove boon to the control programme (Figure 1). In the eradication phase of the programme (1960s), malaria cases were few and far so much so that research activities were abandoned to large extent [2]. It was virtually difficult to locate malaria positive slide for teaching and demonstration purposes in medical colleges and expertise was fast waning. The discipline of 'malariology', however, was revived post-resurgence in 1976 to address the needs of the control programme [3]. In the wake of re-emergence, focal disease outbreaks had become order of the day and routine interventions were not yielding dividends proportional to investments. Recurring costs were escalating and returns diminishing. Given the scenario, alternate interventions were mandated to innovate and field-test newer technologies both against malaria parasite and vectors to contain transmission. In this context, establishment of network of research field stations in

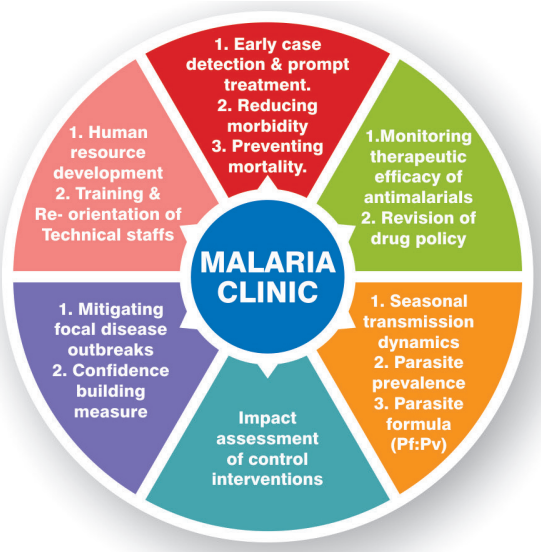

Figure 1. Malaria clinic and benefits to the control programme

the country provided a new boost to the control programme, helping situational assessment in high-risk states and evaluating evidence-based technologies that are community-based and sustainable [4].

One such field station was established in the Sonapur Primary Healthcare Centre, Dimoria Block of Kamrup district of Assam reporting flare up of cases and deaths associated with focal disease outbreaks [5]. Malaria by far was the major public health concern affecting all-round socio-economic development. The indigenous population groups (mostly tribal aborigines) little aware of disease and prevention were adversely affected. In search for tangible solutions, amidst multifaceted approach the first and foremost task was to set up malaria clinic to ensure EDPT helping reposing confidence in the communities at risk (Figure 2). Malaria clinic served pivotal point providing quality services gratis and launching pad helping conduct series of investigations related to disease transmission and control. Enumerated in this chapter are some of the clinic-based research activities that helped understand the local disease epidemiology and institute appropriate interventions to avert continued onslaught of the disease.

True incidence of malaria

Even though state laboratory was attempting to serve best to their capacity, the workload was enormous to cope up with the logistic requirements given the insurmountable morbidity more so during disease outbreaks. Human errors were unescapable in reaching correct diagnosis adding woes to the grieved communities. Additional research facility providing access to prompt diagnosis and treatment served boon to the programme and yielded information all to the benefit of the indigenous populations and control programme. Outdoor patient attendance increased by the day providing services for confirmed diagnosis of malaria gratis. All subjects reporting fever were screened for malaria positivity by examination of finger-prick blood-smear (thick smear) and confirmation of parasite species (thin smear). On occasions more than 100 patients accessed the services of which 30-40 subjects were confirmed positive on any given day by microscopic examination.

Figure 2. Components of the Integrated Disease Vector Control (IDVC) approach for malaria containment. Disease surveillance and treatment were the major stay in combating malaria illness.

The data were tabulated on monthly basis for years in operation and subjected to analyses for relative abundance of parasite species, transmission dynamics and allied investigations (Table 1).

Table 1. Passive case detection for malaria based in the Dimoria block of Kamrup district of Assam (1988-1999), North-East India

Year	No. of fever cases screened for malaria parasite	No. blood-smears +ve for malaria	Smear positivity rate (%)	% smears +ve for *Plasmodium falciparum*
1988	617	212	34.35	79
1989	1475	451	30.57	86
1990	1291	400	30.98	69
1991	3145	1297	41.24	68
1992	4845	1939	40.02	75
1993	5911	2264	38.30	71
1994	8315	3022	36.34	71
1995	11608	4243	36.55	62
1996	6801	2292	33.70	55
1997	4958	1371	27.65	63
1998	3994	1124	28.14	61
1999	7146	2261	31.64	74

Morbidity due to malaria was alarming evidenced by parasite rate that ranged from 27.7% to 41.2% adding penury to poverty-stricken communities. Both *Plasmodium falciparum* and *P. vivax* were abundant, but former was the most predominant infection constituting ≥61% of cases, the remaining were *P. vivax* except sporadic cases of *P. malariae* (rather rare)[6]. Occasionally mixed infections (both *P. falciparum* and *P. vivax*) were recorded but constituted <1% of total cases registered. Invariably, all malaria-attributable death cases were confirmed to be due to *P. falciparum* largely due to delayed reporting (Table 2). Death cases were recorded in all age group of both sexes excluding infants (<1 year of age) representative of low-to-moderate transmission [7].

Table 2. Distribution of malaria-attributable death cases by age and gender during outbreak years based on hospital records in the Sonapur Primary Health Centre (Dimoria Block) of Kamrup district of Assam, North-East India*

Year	Total cases admitted (All diseases)	No. of indoor admissions due to malaria	Deaths due to *Plasmodium falciparum* malaria by age/gender			
			<5 years	5 - 15 years	>15 years	Total (Males/ Females)
1985	997	801	1	2	4	7 (3/4)
1986	1624	1136	4	6	7	17 (10/7)
1991	342	156	1	2	1	4 (1/3)

*State Health Directorate, Government of Assam

Overall, morbidity due to malaria was estimated to be at least four to five times of that what was being reported by the state disease surveillance. Research-based diagnostic services were continuing activity helping reduce morbidity and yielded collateral benefits providing information on seasonal transmission and changing dynamics detailed as below.

Seasonal prevalence of malaria parasite species and transmission dynamics

Malaria cases were recorded during all months inclusive of all age groups suggestive of perennial transmission (Table 3). Year-round transmission was further substantiated by infant parasite rate for all months observed. Relative distribution of cases, however, varied with large concentration in (5-15) years age group compared to all other age groups (P = <0.001). There was substantial rise in cases with the commencement of pre-monsoon showers beginning April up until June, but good number of cases continued to be recorded till September corresponding with the months of rainfall (the peak transmission period), however, following cessation of monsoons there was significant decline in cases for remainder of the year (Figure 3). The rise in cases during peak period was largely attributed to *P. falciparum* (the deadly parasite), whereas *P. vivax* cases exceeded those of *P. falciparum* in low transmission period (dry-season). During peak period, seasonal temperatures ranged from 22^0C to 33^0C and relative humidity remained high (63%-89%). Transmission intensities, however, between years varied significantly (P = <0.0001), yet relative risk of *P. falciparum* infection was low in dry-season (October - March) than wet-season (April - September). Conversely, relative risk of *P. vivax* infection was high in dry-season than wet-season (Table 4). Gametocyte carriage for *P. falciparum*, however, was recorded for all months and varied between 1.31% – 2.16%, but for data based on 1991-1994, it was significantly higher during dry-months than wet-season (P = <0.01) (Figure 4).

Table 3. Monthly distribution of malaria cases by age for data based on passive surveillance in the Sonapur Primary Health Centre (Dimoria Block) of Kamrup district of Assam, North-East India*

Month, 1995	<1 year			1 - <5 years			5–15 years			>15 years			Total		
	BSC	+ ve	Pf	BSC	+ ve	Pf	BSC	+ve	Pf	BSC	+ve	Pf	BSC	+ve	Pf
Jan	3	2	0	18	6	4	55	23	16	184	69	31	260	100	51
Feb	1	0	0	12	5	4	66	19	8	157	36	7	236	60	19
March	1	1	0	22	7	4	65	25	9	200	42	8	288	75	21
April	6	2	0	54	12	5	105	42	26	393	118	56	558	174	87
May	18	9	1	152	59	38	440	234	140	1020	394	237	1630	696	416
June	31	11	6	270	103	77	540	246	194	1396	522	396	2237	882	673
July	15	1	1	172	38	23	338	121	89	1053	355	265	1578	515	378
Aug	22	7	5	104	32	18	238	108	69	644	257	172	1008	404	264
Sept	5	2	2	55	17	9	168	88	60	501	188	113	729	295	184
Oct	8	4	3	63	15	12	168	54	31	431	121	66	670	194	112
Nov	6	2	1	21	8	4	95	46	16	336	130	57	458	186	78
Dec	2	1	0	16	7	4	59	23	12	260	103	37	337	134	53
Total	**118**	**42**	**19**	**959**	**309**	**202**	**2337**	**1029**	**670**	**6575**	**2335**	**1445**	**9989**	**3715**	**2336**

*Malaria Clinic, BSC – blood smears collected, +ve cases are sum of both *Plasmodium falciparum* (Pf) and *P. vivax* (Pv) positive

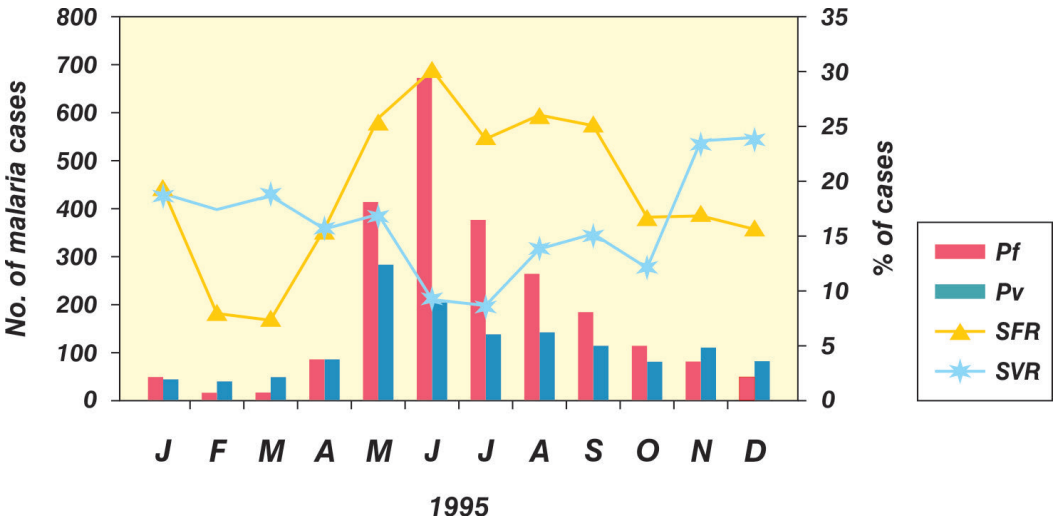

Figure 3. Monthly distribution of cases and parasite rate for data based in the Sonapur Primary Health Centre (Dimoria Block), Kamrup district, Assam, North-East India. Pf, Pv, SFR and SVR denote *Plasmodium falciparum, Plasmodium vivax*, smear falciparum rate and smear vivax rate respectively.

Table 4. Seasonality of malaria parasite species for data based on passive surveillance in the Sonapur Primary Health Centre (Dimoria Block) of Kamrup district, Assam, North-East India*

Year	Dry season (October – March)			Wet season (April – September)		
	No. of blood smears examined	No. (%) of blood smears		No. of blood smears examined	No. (%) of blood smears	
		Positive for *P. falciparum*	Positive for *P. vivax*		Positive for *P. falciparum*	Positive for *P. vivax*
1991	844	186 (22.0)	147 (17.4)	2301	702 (30.5)	262 (11.4)
1992	1376	397 (28.9)	170 (12.4)	3469	1063 (30.6)	309 (8.9)
1993	2090	531 (25.4)	275 (13.2)	3821	1079 (28.2)	379 (9.9)
1994	2219	400 (18.0)	320 (14.4)	6096	1746 (28.6)	656 (10.8)
1995	2249	334 (14.9)	415 (18.5)	7740	2002 (25.9)	964 (12.5)
1996	1887	236 (12.5)	343 (18.2)	4914	1014 (20.6)	686 (14.0)
1997	1144	127 (11.1)	156 (13.6)	3814	732 (19.2)	356 (9.3)
1998	1271	181 (14.2)	163 (12.8)	2723	502 (18.4)	278 (10.2)
1999	1446	204 (14.1)	160 (11.1)	5700	1467 (25.7)	422 (7.4)
2000	1213	137 (11.3)	136 (11.2)	2305	231 (10.0)	189 (8.2)
2001	1067	96 (9.0)	73 (6.8)	2822	467 (16.5)	146 (5.2)
2002	790	82 (10.4)	50 (6.3)	2648	293 (11.1)	197 (7.4)
2003	911	73 (8.0)	40 (4.4)	2043	163 (8.0)	112 (5.5)

*Source Reference [8]

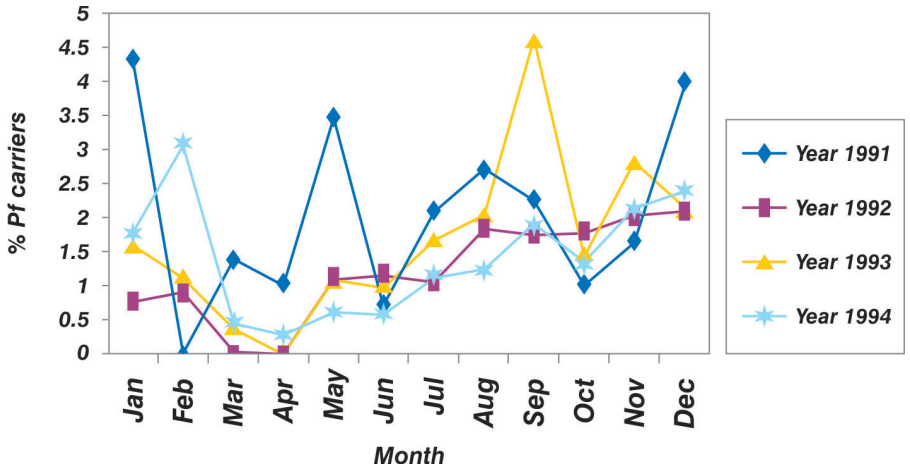

Figure 4. Monthly prevalence of *Plasmodium falciparum* carriers (% smear positives with gametocytes) in the endemic communities for data based on passive surveillance in the Sonapur Primary Health Centre (Dimoria Block), Kamrup district, Assam (1991-1994), North-East India. Source Reference [8]

Relapsing characteristics of *Plasmodium vivax* malaria

Besides, *P. falciparum* (malignant tertian malaria), morbidity due to *P. vivax* (benign tertian malaria) malaria was enormous but often neglected [9]. Transmission of *P. vivax* continued throughout the year and smear positivity rate (SPR) varied from 9% - 24% between months (Figure 3); relative abundance, however, was high during winter/dry-season (October-March). High prevalence of *P.vivax* during these months, in part, could be attributed to its relapsing characteristics of latent hypnozoites. For data based in the study area, relapsing rate was estimated to be 8.8% based on 479 follow up cases assuming that subjects did not comply with the standard primaquine therapy (Table 5). Despite the fact that *P. vivax* malaria is highly susceptible to chloroquine therapy, morbidity is invariably extended due to its inherent biological characteristics of greater resilience in completing sporogony (parasite development in mosquito host) at lower ambient temperatures and multiple relapses months apart. Given the low-to-moderate transmission intensities evidenced by entomological inoculation rates (EIR <1 per person night) [10], and scarce mixed infections of *P. falciparum* and *P. vivax* (<1%), there exists every likelihood of relapsing episode rather than a fresh case of re-infection particularly in dry-periods.

It was observed that there was invariably a second relapse but third and fourth relapsing episodes were less common in decreasing order. Based on cross-sectional surveys in malaria endemic districts of north-eastern states (Table 6), given the similar transmission conditions, a higher proportion of *P. vivax* (Pv) cases consistently occurred among <5-years old compared the proportion of *P.falciparum* (Pf cases) in that age group (P = 0.02).It is hypothesized that high concentration of cases in <5 years might as well be contributed by relapses which were more frequent compared to all other age groups (Figure 5). This study lends to support the hypothesis that *P. vivax* populations in north-east India were typically of tropical type with relapses occurring at short intervals (majority relapses recorded within 90 days) similar to those reported in north India (11). A series of investigations based in various epidemiological zones of India revealed that *P. vivax* populations are

indeed diverse and genetically different inclusive of both relapsing and non-relapsing strains with differing levels of susceptibility to primaquine understanding of which may be of evolutionary significance and for developing effective control interventions [12].

Table 5. Relapsing pattern of *Plasmodium vivax* malaria (treated with chloroquine only) for data based in the Sonapur Primary Health Centre (Dimoria Block) of Kamrup district of Assam (January-December 1992), North-East India*

Jan	Feb	March	April	May	June	July	August	Sept	Oct	Nov	Dec	Total	% Relapse cases
(30)	0	1	2	0	0	0	0	0	0	0	0	3	10
	(15)	1	1	0	0	0	0	0	0	1	0	3	20
		(15)	2	0	1	0	0	0	0	0	0	3	20
			(19)	0	0	1	0	0	0	0	0	1	5.3
				(44)	0	1	0	1	0	0	0	2	4.5
					(52)	0	1	0	0	1	0	2	3.8
						(61)	0	2	3	1	1	7	11.5
							(79)	1	4	0	1	6	7.6
								(54)	6	4	1	11	20.3
									(47)	1	2	3	6.4
										(30)	1	1	3.3
											(33)	-	-
												42	8.8

*Figures in parenthesis are the primary attack cases; Summary statistics: Total study cases: 479, Relapse cases: 42, Relapse rate: 8.8%

Declining transmission trends, and the way forward

Even though all age groups experienced malarial attacks, smear falciparum rate (SFR) was the least in infants (<1year of age) and the highest in (5-15) age group (Figure 6). Similar observations were recorded in other malaria-endemic districts of north-eastern states reporting relatively high proportion of cases in 5-15 age group (Table 6). Consistently, 5-15 age group was the most vulnerable and carried the brunt of the disease burden that was significantly high compared to all other age groups ($P = <0.05$). From the data given for years 1991-2003, regardless the variable number of cases, disease transmission trends were, however, clearly deaccelerating (Figure 7). Nevertheless, while there was steady decline in SPR, *P. falciparum* remained the majority infection. The downward trends continued beyond 2003 (data not shown), during which cases were few and far (SPR<1%) so much so that clinic-based research investigations were abandoned.

Given the rollout of interventions, malaria risk is seen reducing. It is high time to seize the opportunity in upscaling interventions and providing quality services in reaching correct diagnosis and universal treatment access as the parasite becomes scarce.

Table 6. Results of cross-sectional malaria prevalence surveys based on passive case detection in ethnic communities of North-East India*

Location (District, State)	Study period	Age group in years								
		<5			5 –15			>15		
		No. of blood - smears examined	No. and (%) +ve for		No. of blood - smears examined	No. and (%) +ve for		No. of blood - smears examined	No. and (%) +ve for	
			P. falciparum	*P. vivax*		*P. falciparum*	*P. vivax*		*P. falciparum*	*P. vivax*
Diphu (Karbi Anglong, Assam)	August, 1991	43	5 (11.6)	2 (4.7)	49	8 (16.3)	0 (0)	87	11 (12.6)	0 (0)
Rangapara (Sonitpur, Assam)	May – June, 1992	409	86 (21.0)	12 (2.9)	221	72 (32.6)	16 (7.2)	441	139 (31.5)	50 (11.3)
Panerihat (Udalguri, Assam)	Aug – Sept., 1992	604	269 (44.5)	56 (9.3)	688	351 (51.0)	66 (9.6)	1661	798 (48.0)	117 (7.0)
Koilamari (Lakhimpur, Assam)	June – July, 1994	230	61 (26.5)	13 (5.7)	411	178 (43.3)	33 (8)	798	340 (42.6)	54 (6.8)
Doomdoma (Tinsukia, Assam)	Sept., 1994	102	8 (7.8)	2 (2.0)	158	17 (10.8)	10 (6.3)	443	45 (10.2)	11 (2.5)
Umrangsu (N.C. Hills, Assam)	Oct., 1994	4	1 (25.0)	0 (0)	4	1 (25.0)	0 (0)	55	11 (20.0)	3 (5.5)
Sonapur (Kamrup, Assam)	Jan – Dec, 1995	1077	221 (20.5)	130 (12.1)	2337	670 (28.7)	359 (15.4)	6575	1465 (22.3)	860 (13.1)
Goreshwar (Kamrup, Assam)	March, 1995	22	2 (9.1)	1 (4.5)	55	5 (9.1)	0 (0)	89	9 (10.1)	5 (5.6)
Agia (Goalpara, Assam)	April – May, 1995	293	92 (31.4)	61 (20.8)	741	286 (38.6)	115 (15.5)	1263	409 (32.4)	131 (10.4)
Jamaguri (Sonitpur, Assam)	May, 1995	250	58 (23.2)	25 (10.0)	634	134 (21.1)	74 (11.7)	997	214 (21.5)	124 (12.4)
Mazbat (Darrang, Assam)	July, 1996	259	41 (15.8)	13 (5.0)	605	114 (18.8)	17 (2.8)	792	130 16.4)	27 (3.4)
Hamren (Karbi Anglong, Assam)	Aug., 1996	14	3 (21.4)	1 (7.1)	47	16 (34.0)	1 (2.1)	125	20 (16.0)	2 (1.6)
Nellie (Morigaon, Assam)	July – Aug., 1999	84	26 (31.0)	7 (8.3)	191	81 (42.4)	11 (5.8)	352	141 (40.0)	8 (2.3)
Boginadi (Lakhimpur, Assam)	April, 2006	204	31 (15.2)	43 (21.1)	469	95 (20.3)	59 (12.6)	793	133 (16.8)	72 (9.1)
Golaghat (Golaghat, Assam)	April, 2006	111	10 (9)	4 (3.6)	444	39 (8.9)	8 (1.8)	537	32 (6.0)	1 (0.2)
Dalu (West Garo Hills, Meghalaya)	May – June, 2007	283	46 (16.3)	12 (4.2)	450	86 (19.1)	18 (4)	403	39 (9.7)	6 (1.5)
Tlabung (Lunglei, Mizoram)	May – Aug., 2012	228	36 (15.8)	2 (0.9)	278	67 (24.1)	8 (2.9)	447	85 (19.0)	0 (0)
Silachari (Gomti, Tripura)	July – Sept., 2012	43	4 (9.3)	1 (2.3)	55	8 (14.5)	0 (0)	124	19 (15.3)	1 (0.8)

*Source Reference [9]

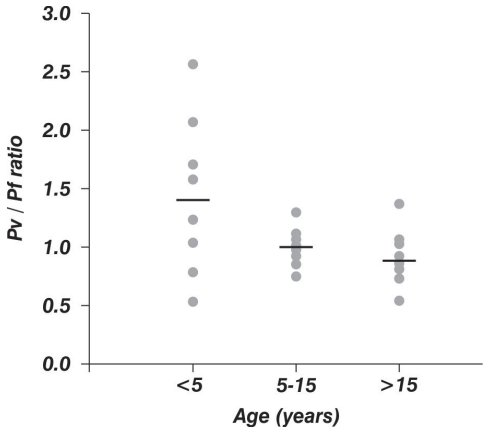

Figure 5. Relapsing characteristics by age groups for data based in malaria-endemic districts of north-eastern states of India. The graph shows the ratio of the percent of *Plasmodium vivax* (Pv cases) in an age group with the percent of *Plasmodium falciparum* (Pf cases) in the same age group for a given study in each of 10 study sites (rest were excluded due to small sample size) given in Table 6. The bar represents the mean ratio (Credits: Naman K Shah, Department of Epidemiology, University of North Carolina, USA).

Conclusions

The presented data were based on gold standard microscopy results giving information on prevalence of malaria, parasite species abundance and seasonal transmission dynamics. However, many cases possibly were missed especially sub-patent parasitaemia (asymptomatic low-density infections) for which molecular techniques would have been an added advantage. Asymptomatic infections are of common occurrence in endemic settings and often go undetected/untreated having wider implications in malaria elimination [8,13]. PCR-based assays can prove to be a handy tool revealing not only sub-patent parasitaemia but also mixed infections which were normally missed in routine microscopic examination of peripheral blood-smears [14-16]. Zoonotic malaria, an emerging paradigm,

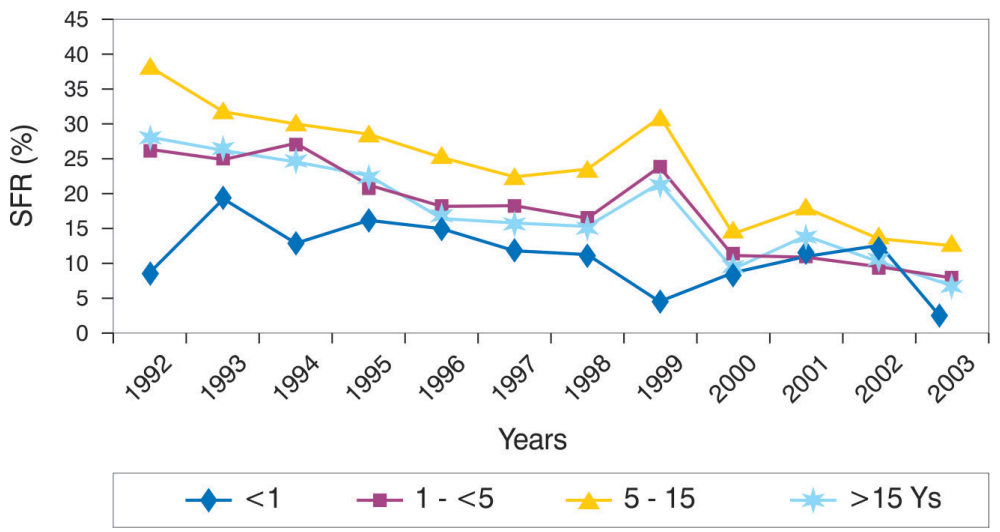

Figure 6. Relative contribution of *Plasmodium falciparum* cases by age group for data based on passive surveillance in the Sonapur Primary Health Centre (Dimoria Block), Kamrup district, Assam, North-East India. SFR denotes % smear positives for falciparum infection. Source Reference [8]

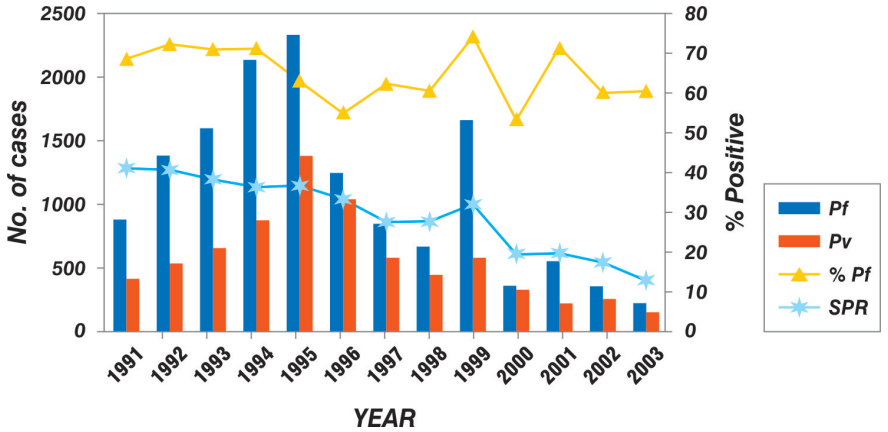

Figure 7. Declining transmission of malaria for data based on passive surveillance in the Sonapur Primary Health Centre (Dimoria Block), Kamrup district, Assam (1991-2003), North-East India.Pf, Pv and SPR denote *Plasmodium falciparum*, *Plasmodium vivax* and smear positivity rate respectively. Source Reference [8]

which is making inroads in neighbouring Southeast Asian countries [17,18], perhaps also exits in northeast but no records for lack of requisite expertise and infrastructure (presently available in central laboratories) at the block level of control operation. Nevertheless, malaria clinic services proved boon to the control programme in many ecological settings not only providing prompt diagnosis and treatment but also helped in understanding local disease epidemiology and building confidence in the communities at stake [19]. Clinic-based studies generated evidence helping implement control interventions both against mosquito vector to guard building densities as well as causative parasite in upgrading drug-policy for radical cure averting impending disease outbreaks. Given the declining transmission trends in the Northeast, clinic-based services should be further strengthened by: (i) augmenting laboratory services with molecular diagnostics, (ii) training and re-orientation of the laboratory service personnel, (iii) prioritizing laboratory services in high-risk communities at sub-centre level, (iv) ensuring universal access for quality health services to all those in need irrespective of financial and legal status. In addition, availability of skilled staff strength, which is fast depleting must be heeded to for continued services uninterrupted. These measures would go long way in addressing programme needs to avert return of drug-resistant malaria especially post-elimination [20].

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7

Tryst with malaria outbreaks: genesis and containment strategies

Introduction

North-East (NE) India is highly receptive for malaria transmission due to favourable determinants including extended rainy season, innumerable mosquito breeding habitats, conducive temperatures supporting sporogony (parasite development in the mosquito host), multiple vector species and the inhospitable terrain limiting access to healthcare services [1]. All seven sister states of NE India (including Arunachal Pradesh, Assam, Manipur, Mizoram, Meghalaya, Nagaland, Tripura) are co-endemic for both *Plasmodium falciparum* and *P. vivax* malaria; relative abundance of parasite species, however, varied between states [2]. Collectively, NE states contributes substantial number of cases annually most of which are due to *P. falciparum* (the deadly parasite). Disease transmission intensities are estimated to be low-to-moderate but perennial in large tracts of land including valleys and foothills except elevations beyond 3000 feet above msl. The relative risk of malaria, however, varies between endemic districts in relation to geographic location of human settlements, vector breeding habitat and distance from healthcare facility [3,4]. All NE states are prone to focal disease outbreaks characterized by marked rise in cases and malaria-attributable deaths manifold. Included in this chapter is an account of malaria outbreaks investigated and specific recommendations helping the national control programme strengthening interventions to mitigate the risk of disease onslaught in place and time.

Malaria outbreaks in Assam

Assam (the most populous state of NE India) has long history of malaria outbreaks which were largely focal in nature affecting groups of villages generally located far away from town areas having poor access to healthcare services reporting sudden support of cases and attributable deaths [5,6]. The state of Assam shares international border with Bhutan and Bangladesh as well as interstate border with all sister states of NE region (Figure 1). Invariably, populations adjoining to the interstate/international border/forest-fringe were adversely affected and the patient load, malaria being the major public health illness, was way too high to cope up by the nearest healthcare facility [7-9]. Hordes of patients reporting fever were seen queuing for getting tested for malaria parasite and those with severe complications requiring inpatient hospital care (Figure 2). Panic and chaos prevailed in the affected populations and the communities lacked communication means, resources and awareness on disease prevention and treatment. During the past two decades, segments of indigenous communities, proportions of which varied anywhere between 33% - 39%, faced wrath of malaria upsurge during the years 1999, 2001 and 2006 as revealed by state health services data reporting heightened morbidity and associated mortality (Table 1). Given the interventions in force and coverage of risk-populations, malaria-attributable

deaths were observed each year between 1998-2008. In 2006, 22 of 23 districts reported malaria-attributable deaths, each microscopically confirmed, due to *P. falciparum* infection related complications (Table 2); the actual number of deaths, however, is estimated to be manifold for many more cases gone unreported and/or for non-availability of confirmed cause of death; several cases even had the least opportunity for having access to healthcare services. Of 304 deaths on record in 2006, majority were reported in districts of Lakhimpur and Golaghat (both sharing interstate border with Arunachal Pradesh and Nagaland respectively) having populations living in forest-fringe areas, which were subject to investigations to contain disease outbreak and spread. Salient observations are detailed as below.

Figure 1. Map of the eastern and North-Eastern states of India showing state boundaries and international border with Myanmar to the east, Bangladesh to the south, Bhutan to the west, and China (Tibet) to the north. Source: North-East Wikipedia.

Figure 2. A view of the healthcare facility to its fullest capacity with indoor admissions due to malaria outbreak. Hospital beds were seen shared by two patients each for want of beds requiring medical attention.

Table 1. Malaria transmission intensities and vector control interventions in Assam, North-East India (1998 – 2008)*

Year	Population in millions (% pop affected)**	No. and (%) of blood-smears			% positive blood - smears with *Plasmodium falciparum*	Annual parasite incidence (no. of confirmed cases / 1000 population)	DDT intervention data***				No. of death cases
		Examined (% of population checked)	Positive for malaria parasite (%)	Positive for *Plasmodium falciparum* (%)			Targeted population (% coverage)		Quantity used in Kg		
							1st spray	2nd spray	1st	2nd	
1998	26.12	2441084 (9.34)	94645 (3.87)	54769 (2.24)	57.86	3.61	14658872 (79.67)	Not done	877904	0	34
1999	26.63	2872859 (10.78)	131048 (4.56)	83064 (2.89)	63.38	4.91	15436286 (81.86)	6031876 (76.90)	1081477	363830	111
2000	26.90	2215375 (8.23)	84915 (3.83)	52116 (2.35)	61.37	3.15	14592590 (79.30)	6967822 (61.89)	937238	339453	43
2001	27.32 (39)	2432620 (8.90)	95142 (3.91)	58951 (2.48)	61.97	3.48	15645797 (77.02)	Not done	917766	0	122
2002	27.73 (23)	2325105 (8.32)	89601 (3.85)	55825 (2.40)	62.30	3.21	14008712 (70.80)	5838094 (70.63)	823537	292956	72
2003	27.85 (17)	2133820 (7.66)	76570 (3.85)	48647 (2.27)	63.53	2.74	14144089 (77.45)	7071625 (90.00)	802885	378449	53
2004	28.73 (13)	1853560 (6.45)	58134 (3.13)	41400 (2.23)	71.21	2.02	11842156 (81.00)	Not done	748801	0	52
2005	29.00 (31)	2050261 (7.06)	67885 (3.31)	45453 (2.21)	66.95	3.34	12964867 (78.00)	Not done	797253	0	113
2006	29.32 (33)	2743092 (9.35)	126178 (4.59)	82546 (3.00)	65.42	4.30	12534987 (74.00)	12820065 (91.42)	756540	1381905	304
2007	29.65 (30.71)	2399836 (8.09)	94853 (3.12)	65542 (2.73)	69.09	3.19	13365385 (81.72)	109297784 (75.21)	840431	575991	152
2008	30.59 (8.62)	2687756	83939 (3.12)	58124 (2.20)	69.30	2.74	8427442 (79.00)	7696344 (78.00)	495373	428912	86

*Source Reference [10]; **% population reporting high rise in cases and malaria-attributable deaths; ***Two successive rounds of DDT indoor residual sprays were scheduled each lasting six weeks.

Parasitological observations

Passive case detection in affected populations of these districts revealed high malaria positivity with gametocyte carriage in all age groups with preponderance of *P. falciparum* cases, the remaining were due to *P. vivax* (Table 3,4). Parasite prevalence and relative abundance of parasite species, however, varied between districts. While parasite positivity in clinical cases was high in Lakhimpur (433/1466, 30%), it was much less in Golaghat (94/1092, 9%), majority cases were due to *P. falciparum* (93%) in both these locations. Introspection of data among reported death cases revealed that majority (44%) of those admitted in the hospital died the same day of admission (apparently late reporting), while 39% died within 2 days, 14% within 3 days of hospital care, and 3% in ≥3 days of reporting (Source: Chief Medical & Health Officer, Lakhimpur). It was observed that of all admissions in Civil Hospital of Lakhimpur town, 85% were due to malaria alone with severe complications. The cure rate, however, was 99%; only 1% succumbed to death.

Table 2. Population affected and malaria-attributable mortality during outbreak year 2006 in Assam state (status report up to June 2006), North-East India

District	Outbreak affected areas			Interventions		Malaria - attributable deaths (confirmed + suspected)**
	Population (% of total)	PHCs*	Villages	Population coverage under DDT	Health camps	
Lakhimpur	93264 (10)	3	162	353597	519	76 (76 + 0)
Golaghat	99010 (10)	5	202	20333	251	45 (38 + 7)
Sonitpur	86095 (4)	8	49	528754	192	12 (9 + 3)
Morigaon	15350 (2)	3	8	273183	106	14 (6 + 8)
Nagaon	1870 (0.1)	1	265	307291	217	5 (5 + 0)
Jorhat	59463 (5)	2	38	328123	0	6 (6 + 0)
Darrang	2444 (0.2)	2	4	483498	187	11 (5 + 6)
Goalpara	1486 (0.2)	1	0	30881	0	4 (3 + 1)
Nalbari	18370 (2)	2	16	205760	3	25 (6 + 19)
Kamrup	458528 (14)	15	512	365694	59	22 (22 + 0)
Barpeta	No data	5	0	15150	17	7 (7 + 0)
Dhemaji	31692 (16)	4	436	149126	0	15 (11 + 4)
Dhubri	4299 (0.3)	3	-	189954	0	3 (3 + 0)
Bongaigaon	267671(28)	0	0	367568	17	4 (1 + 3)
Hailakandi	No data	-	-	18696	-	2 (2 + 0)
Karbi Anglong	40175 (5)	6	28	332778	53	30 (28 + 2)
Kokrajhar	No data	-	-	96782	24	5 (5 + 0)
Sibsagar	No data	-	-	53445	-	1(1 + 0)
Karimganj	No data	-	-	13824	25	1(1 +0)
N.C. Hills	No data	-	-	16501	0	1(1 + 0)
Tinsukia	No data	-	-	48106		2 (2 + 0)
Dibrugarh	No data	-	-	45752		2 (2 + 0)
Total	**1123761 (4)**	**66**	**1720**	**4262582**	**1702**	**292 (239 + 53)**

*PHC denotes Primary Health Centre; **clinical cases of malaria for which blood-smear report was not available

Among those died of malaria, the most common complications included hepatic jaundice, kidney failure and cerebral involvement associated with severity of *P. falciparum* infection in decreasing order of their occurrence. Invariably, in Assam state the rise in *P. falciparum* cases corresponded well with rise in attributable death cases during the period of 1991 - 2008 (Figure 3). Deaths, however, were recorded in all age groups of both sexes; often number of deaths were significantly higher in males than females (Table 5).

Table 3. Passive case detection of malaria in Boginadi Primary Health Centre, Lakhimpur district of Assam, North-East India*

Age group in years	No. blood smears examined for malaria parasite	No. (%) blood smears positive for malaria**	*Plasmodium falciparum* cases***		% *Plasmodium falciparum* of total +ve cases
			Pfr	Pfg	
< 1	24	4 (17)	1	0	25
1 - <5	180	70 (39)	30	1	44
5 - 15	469	154 (33)	95	8	67
> 15	793	205 (26)	133	9	69
Total	1466	433 (30)	259	18	64

*Study period: April 16 – 26, 2006, ** includes both *Plasmodium vivax* and *P. falciparum* cases, *** Pfr denotes *P. falciparum* with ring stage; Pfg denotes *P. falciparum* with both rings + gametocytes.

Table 4. Passive case detection in malaria outbreak affected areas of Golaghat district of Assam, North-East India*

Age group in years	No. blood smears examined for malaria parasite	No. (%) blood smears positive for malaria**	*Plasmodium falciparum* cases***		% *Plasmodium falciparum* of total +ve cases
			Pfr	Pfg	
<5	111	14 (13)	10	1	79
5 - 15	444	47 (11)	39	5	96
>15	537	33 (6)	32	0	97
Total	1092	94 (9)	81	6	93

*Study period: April 15 – 24, 2006; ** includes both *Plasmodium vivax* and *P. falciparum* cases, *** Pfr denotes *P. falciparum* with ring stage; Pfg denotes *P. falciparum* with both rings + gametocytes.

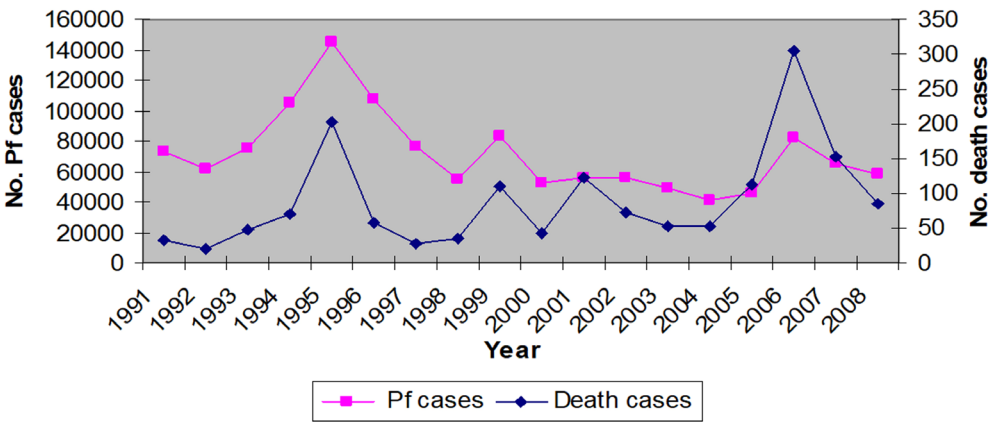


Figure 3. Malaria-attributable morbidity due to *Plasmodium falciparum* (Pf) cases and deaths in Assam during 1991 – 2008 (Source, State Health Directorate of Assam); Source Reference [10]

Table 5. Distribution of malaria-attributable deaths by age and gender during 2007-2009 in Assam, North-East India*

Reporting Year	(<5) yr		(5 – <9) yr		(9 – <15) yr		(≥15) yr		Total cases	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
2007	13	08	07	12	11	12	55	34	86	66
2008	11	10	06	03	04	06	32	14	53	33
2009	09	04	04	04	04	04	22	08	39	20
Total	**33**	**22**	**17**	**19**	**19**	**22**	**109**	**56**	**178**	**119**

*Source Reference [10]

Entomological observations

During 1988-2006, entomological investigations in various malaria outbreak affected districts revealed the abundance of *Anopheles minimus* (the major vector in NE India) and were incriminated by detection of motile sporozoites in salivary glands (Table 6). Mosquito biting rate (MBR) varied between 12-34 per person night but entomological inoculation rate (EIR) remained <1 suggestive of low-to-intermediate transmission. The intensities and risk factors were representative of heterogenous transmission across the state yet EIRs were significantly correlated with high positivity due to *P. falciparum* infection in clinical cases and reported deaths. The role of another efficient vector, *An. baimaii* (member species of the *An. dirus* complex) could not be clearly established in valleys but has been substantiated in supplementing transmission particularly in forest-fringe population groups/forest-dwellers and shifting cultivation harvesters resulting in statistically higher number of deaths in males than females [11].

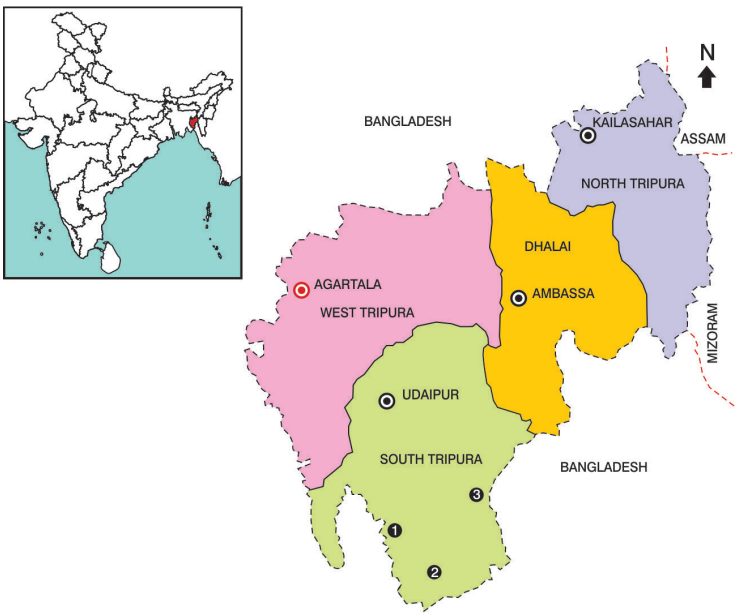
Table 6. Abundance of *Anopheles minimus*, sporozoite infectivity, mosquito biting and entomological inoculation rates, and prevalence of malaria in various districts of Assam state reporting focal disease outbreaks*

District	Location	Study Period	Daytime resting collections per person hour	Mean biting/ rate/ person/ night (MBR)**	No. mosquitoes dissected (sporozoite rate)	EIR*** (MBR x sporozoite rate) per person/ night	% Positivity in clinical cases of malaria	
							Plasmodium falciparum	*P. vivax*
Kamrup	Sonapur	Jun. - Oct. 1988	07.00	14.00	332 (0.033)	0.46	17.0	11.0
Sonitpur	Rangapara	Jul. - Sep. 1992	02.00	13.00	142 (0.042)	0.55	42.8	10.2
Darrang	Tangla	Aug. - Sep.1992	11.00	20.00	382 (0.031)	0.62	48.0	8.1
Golaghat	Bokakhat	May - Jul. 1994	05.35	12.25	303 (0.010)	0.12	6.2	22.8
Goalpara	Agia	Apr. - May 1995	0.95	18.75	105 (0.029)	0.54	34.5	13.4
Morigaon	Nellie	Jul. - Aug. 1999	02.87	23.00	130 (0.031)	0.71	39.4	4.1
Lakhimpur	Boginadi	Apr. - Jun. 2006	07.00	34.00	No data	-	18.9	10.6

*Source Reference[4], **MBR = Mosquito Biting Rate; ***EIR = Entomological Inoculation Rate

Malaria outbreaks in Tripura

Even though all states of NE India are prone to malaria outbreaks, yet the state of Tripura is uniquely placed for sharing vast international border (84% of total border length) with Bangladesh. It is a landlocked state with large concentration of tribal populations along the international border at high-risk of malaria having poor access to treatment. Mosquito fauna and transmission dynamics are akin to Assam, but *P. falciparum* is far more abundant constituting >90% of total cases in the state [12]. Disease distribution, however, was uneven with large concentration of cases in South Tripura and Dhalai districts coinciding with the vast forest cover and tribal aborigines (Figure 4).



District	Pop. in millions (% scheduled tribes pop.)	Forest cover in Sq. Km.	No. of Malaria Cases
North Tripura	0.693 (22)	1223	491 - 2331
West Tripura	1.724 (22)	1146	1001 - 5021
Dhalai	0.398 (44)	1859	2766 - 7658
South Tripura	0.878 (33)	2066	7261 - 13519

Figure 4. Map of Tripura state showing study locations in South Tripura district, (1) Hrishyamukh [23^0 7′ 40.46″ N, 91^0 31′ 23.82″ E], (2) Manubankul [23^0 8′ 26.24″ N, 91^0 42′ 0.93″ E], (3) Silachari [23^0 13′ 51.49″ N, 91^0 46′ 28.47″ E] blocks in proximity to Bangladesh international border. Inset is the map of India showing geographical location of Tripura marked in red. Inset table gives the demographic information and range of reported malaria cases during 2008-2012 in each district of Tripura state (data source, State Health Directorate of Tripura). In 2012, these four districts were further split to present strength of eight.

Malaria transmission is perennial and attributable deaths are reported invariably each year. In 2014, state experienced an outbreak of large magnitude costing 96 lives (deaths spread across all age groups of both sexes), highest ever in the past few decades, solely attributed to *P. falciparum* malaria (Source: State Health Directorate). As much as 30% of the state population, mostly living in remote localities with poor access to treatment, was adversely affected. Point prevalence studies during June-July 2014 across districts revealed

high parasite rate in Dhalai and South Tripura districts with predominance of *P. falciparum* cases (Table 7). Earlier in 2012, cross-sectional malaria surveys in malaria-endemic blocks of South Tripura revealed similar results showing high positivity in clinical cases that varied from 26% in Silachari to 53% in Hrishyamukh (Table 8). Entomological investigations in these study blocks revealed prevalence of both *An. minimus* and *An. baimaii*, of which the former was incriminated by demonstrating sporozoites in salivary glands, however, EIR was <1% (Table 9). Apparently, *An. minimus* played major role in malaria transmission having eco-biological characteristics similar to that of Assam of resting indoors (Figure 5). Nevertheless, CDC light trap collections in several places in Tripura revealed greater abundance of *An. baimaii* (forest dweller species) and were incriminated by PCR-based assays for high sporozoite infectivity of *P. falciparum* [13]. It is an outdoor resting species yet have strong predilection entering dwellings for human blood meal.

Table 7. Point prevalence of malaria in districts of Tripura, North-East India (June-July 2014)

District	Forest cover in Sq Km	Pop. in millions (% ST population)	No. of blood-smears examined	No. and (%) +ve for	
				Plasmodium falciparum	*Plasmodium vivax*
North Tripura	1223	0.693 (22)	259	32 (12.3)	1 (0.5)
West Tripura	1146	1.724 (22)	643	22 (3.4)	0
Dhalai	1859	0.398 (44)	742	207 (27.9)	1 (0.1)
South Tripura	2066	0.878 (33)	80	38 (47.5)	7 (8.7)
Total	**6294**	**3.810 (31)**	**1724**	**299 (17.3)**	**9 (0.5)**

Table 8. Results of cross-sectional malaria prevalence surveys in malaria endemic blocks of South Tripura district, North-East India*

Study location (Sub - division)	Population surveyed	Study period	Type of collection	No. of blood-smears examined	Fever rate (%)	No. positive for malaria parasite		
						Plasmodium falciparum	*Plasmodium vivax*	Total cases (% smear +ve)
Manubankul (Sabroom)	2860	June – July 2012	Afebrile	561	12	0	0	0
			Febrile	76		31	1	32 (42)
Harishya-mukh (Belonia)	4086	June – July 2012	Afebrile	716	21	5	3	8 (1)
			Febrile	196		91	12	103 (53)
Silachari (Karbook)	3474	Sept – Dec., 2012	Afebrile	655	11	26	4	30 (5)
			Afebrile	80		20	1	21 (26)
All sites	**10420**	**Total**	**Afebrile**	**1932**	**15**	**31**	**7**	**38 (2)**
			Febrile	**352**		**142**	**14**	**156 (44)**

*Source Reference [12]

Table 9. Mosquito landing rates, sporozoite positivity and transmission intensities of malaria in different blocks of South Tripura district, North-East India

Study location (Sub - division)	Study period	Mosquito species	Mean mosquito landing rate per person night*	No. of mosquitoes dissected (sporozoite infection rate)	EIR (mosquito landing rate x sporozoite infection rate)**	% Positivity in clinical cases of malaria	
						Plasmodium falciparum	*P. vivax*
Hrishyamukh (Belonia)	June-July, 2012	*An. minimus*	8.25	37 (0.054)	0.44	46.4	6.1
		An. baimaii	6.00	14 (0)	0		
Manubankul (Sabroom)	July, 2012	*An. minimus*	0.50	4 (0)	0	40.7	1.3
		An. baimaii	4.00	10 (0)	0		
Silachari (Karbook)	Sept-Dec., 2012	*An. minimus*	3.25	20 (0.05)	0.16	25.0	1.3
		An. baimaii	0	0	0		

*Data based on 4 person nights at each location; **EIR = Entomological Inoculation Rate; Source Reference [12]

Figure 5. Resting habitats of *Anopheles minimus*; left – house made of split bamboo with thatched roofing is an ideal resting habitat; right – hanging articles within house premises are the preferred resting sites.

The role of other mosquito species, *An. jeyporiensis* in particular, could not be ruled out contributing to transmission for having bionomical characteristics similar to that of *An. minimus* [12].

Genesis of malaria outbreaks and containment practices

Assam

The malaria control strategy implemented by the Indian National Vector Borne Diseases Control Programme largely rests on two main pillars, i.e., (i) disease surveillance and treatment, (ii) indoor residual spraying for vector containment. Both these interventions remained inadequate due to missing of 2nd round of spray coverage in several years, and disease surveillance that can be best described as fragmented being <10% of population

screened years together (Table 1). There was dearth of intermediate supervision on both these counts resulting in build-up of vector densities and reservoir of asymptomatic parasitaemia evidenced by gametocyte carriage in endemic communities (Table 3, 4). It was apparent from the data (Figure 3) that there was a spike in malaria cases after every few years marked by substantial increase of *P. falciparum* (deadly parasite) on account of outbreaks. Most of these outbreaks were focal in nature affecting marginalized population groups living in forest-fringes along the interstate and/or international border areas with poor access to treatment. Apparently, there was unusual build-up of vector density on account of neglected vector control operations for years together resulting in increased propensity of disease transmission. Stratification based on annual parasite incidence (API) data for the period 2004-2008 in Assam revealed that areas with low endemicity (API <1) had turned hyper-endemic (API >10) due to lack of adequate interventions and consequent increased receptivity, where as districts with moderate endemicity (API 1-4 and 4-10) remained static with little variation (Figure 6).

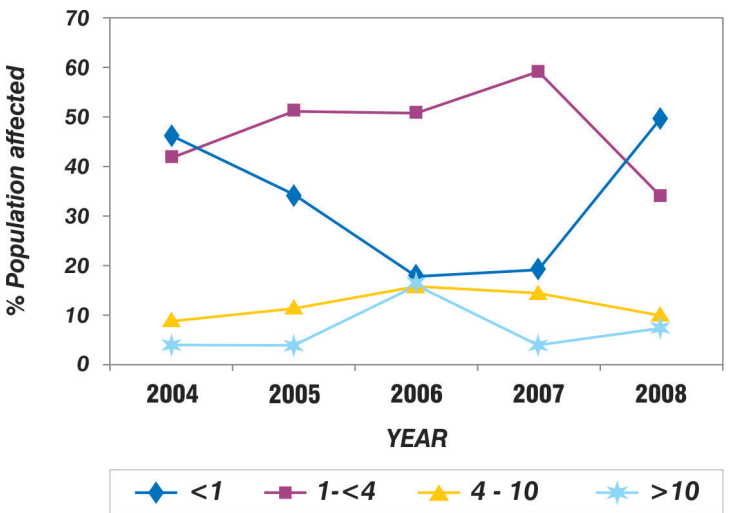


Figure 6. Malaria stratification based on annual parasite incidence per thousand population for districts of Assam during 2004–2008. Source Reference [12]

In Assam, areas sharing international border with Bhutan, and interstate border with Arunachal Pradesh and Nagaland were particularly vulnerable having vast stretches of reserve forests and indigenous populations living under impoverished conditions having poor communication means and little awareness on disease prevention and treatment (Table 10). Situational analyses in these areas revealed that while disease surveillance was far from satisfactory(<10% of population screened), indoor residual spray was either non-existent or remained patchy with <50% coverage of the targeted population [14]. The district health authorities were not prepared to meet the exigencies on account of logistic shortfall of antimalarials and attrition of skilled workforce. There was gross under-reporting of cases amounting to poor response to arising imminent threats perpetuating vicious cycle of malaria and poverty. Invariably these pockets were adversely affected by incessant rains and waves of flash-floods and marred with insurgent activities/social unrest restricting access to the communities at risk.

Table 10. Malaria transmission in population settlements along interstate and international bordering districts of Assam, North-East India for epidemiological data based on 2001*

Interstate/ international border	No. of bordering districts (PHCs)	Population of bordering PHCs	No. of blood-smears examined for malaria parasite (% of population checked)	No. and (%) of blood-smears		% of malaria cases positive for *Plasmodium falciparum*	No. of death cases
				+ve for malaria parasite (SPR)	+ve for *Plasmodium falciparum* (SFR)		
Indo-Bhutan	6 (12)	2265137	195215 (8.6)	14634 (7.5)	5008 (2.6)	34	10
Indo-Bangla	3 (10)	1943795	84982 (4.4)	1316 (1.5)	1215 (1.4)	92	2
Arunachal	7 (19)	4138566	246669 (5.9)	19536 (7.9)	4661 (1.9)	24	26
West Bengal	2 (5)	1043973	43801 (4.2)	1366 (3.1)	967 (2.2)	71	5
Manipur	2 (5)	612617	21776 (3.5)	1075 (4.9)	1058 (4.8)	98	0
Mizoram	3 (5)	1145594	44176 (3.8)	1766 (4.0)	1746 (3.9)	99	2
Meghalaya	7 (19)	3028263	148434 (4.9)	8765 (5.9)	7181 (4.8)	82	7
Nagaland	5 (13)	2161451	148116 (6.8)	6659 (4.5)	5747 (3.9)	86	22
Tripura	1 (1)	293445	21897 (7.4)	126 (0.57)	101 (0.5)	80	0

*State Health Directorate of Assam; Source Reference [15]

Tripura

The scenario is no different for the state of Tripura sharing vast border with Bangladesh. Besides inadequate interventions in place and time (missing rounds of residual spray operations and patchy distribution of mosquito nets), the level of technical expertise was adjudged to be grossly inapt for providing diagnosis resulting in inadequate treatment. There was huge problem of reaching correct identification of malaria parasite species leaving many cases untreated while others received wrong prescription (Table 11). There was virtual lack of data on therapeutic efficacy of antimalarial drugs in force for treatment of malaria, while entomological information on prevalent vector species and insecticide susceptibility status was non-existent.

Table 11. Cross-checking of malaria test reports of the state laboratory technicians based in Tripura, North-East India (June – July 2014)

District	No. of hospitals visited	No. of blood-smears cross-checked	
		No. +ve smears (% discrepancy)	No. –ve smears (% discrepancy)
West Tripura	5	50 (22)	50 (8)
Dhalai	8	50 (16)	50 (36)
South Tripura	1	10 (10)	10 (80)
North Tripura	5	50 (10)	50 (6)
Total	**19**	**160 (16)**	**160 (21)**

Focal outbreaks of malaria came to light only through media alarmed by unusual rise in death cases creating panic in affected communities. There appeared to be no vigilance mechanism/early warning system in place for detecting malaria outbreak by concerned district authorities. Besides, there was dearth of supervision at all levels of operation and response was too little and too late. To contain the disease spread, usual response included

setting up malaria clinics (health camps) providing on-the-spot diagnosis and treatment as well as additional round of residual spraying prioritizing affected population groups. Amidst hue and cry, research organizations and NGOs came into action to augment diagnostic services and supplement interventions through mosquito net distribution as well as impregnation of community-owned nets with insecticide for personal protection helping contain the disease outbreak restoring confidence in risk communities.

Specific recommendations

To sum up, the larger issue in NE India is the cross-border malaria/forest malaria that requires serious attention not only to contain focal outbreaks but also to arrest the development and spread of drug-resistant strains. Human settlements in vulnerable pockets (erstwhile reserve forest areas) are swelling on account of illegal migration across the borders which are at increased risk of infection for lack of interventions coupled with poor access to healthcare services. There is a need for strengthening infrastructure in reaching out these marginalized population groups for which cross-border initiative between participating governments is mandated in meeting the logistics and coordinated action to mitigate the disease onslaught [16]. The data sharing mechanism should be in place for existing drug-treatment policy of malaria cases as well as susceptibility status of disease vectors helping schedule spraying operations in time and place. Population movement across borders should be guarded through check-posts strengthened with treatment and diagnostic facility for preventing dissemination of drug-resistant malaria strains. Apart from government aided programme in healthcare delivery services, role of non-governmental organizations/philanthropist/faith-based organizations should also be explored for augmenting preventive measures and access to early case detection and treatment in these outreach population groups. Strengthening entomological capacity is mandated for generating data on disease vectors which are crucial to formulate policy in view of the fast-changing ecological context and climate change to avert possible disease outbreaks helping instituting interventions to check vector populations [17,18]. Training and re-orientation programmes of field workers/technicians/fresh recruits should be the continuing activity to help upgrade their skills and to overcome human resource attrition. Heath education and behaviour change communication should be the guiding principles to elicit community participation which would go a long way in increased community compliance for seeking treatment seeking well in time. Given the present-day knowledge on bionomics of disease vectors and parasite biology [19], disease transmission trends are clearly deaccelerating [20], yet much more can be achieved by prioritizing interventions in these pockets to conquer malaria paving way to achieve malaria elimination by 2030 [21].

The way forward

The rollout of newer interventions namely insecticide-treated nets (ITNs)/long-lasting insecticidal nets (LLINs) and artemisinin-based combination therapy (ACTs) have resulted in sea change in reducing transmission risk in areas once considered intractable (Figure 7). Malaria outbreaks are rare and case incidence is deaccelerating surely and

steadily for the past few years. Additional provision of staffs under National Health Mission (NHM) and increased allocation under Global Fund to fight Aids, Tuberculosis and Malaria (GFATM) have helped bridging the gap in mitigating the risk of infection. Induction of Accredited Social Health Activist (ASHA) workers has gone a long way in ensuring early malaria case detection and treatment in communities reducing morbidity. Disease surveillance should be further strengthened by induction of new point-of-care tools such as battery-operated magneto-optical devices and DNA aptamer-based techniques helping test-track-treat to delay emergence of drug-resistant malaria [22]. Apparently, there are many more cases left untreated on account of sub-patent/low-density parasitaemia which must be attended to disrupt transmission. It is time to deliver accelerating equitable access to better quality primary healthcare services aiming universal coverage in communities at any risk [23,24].

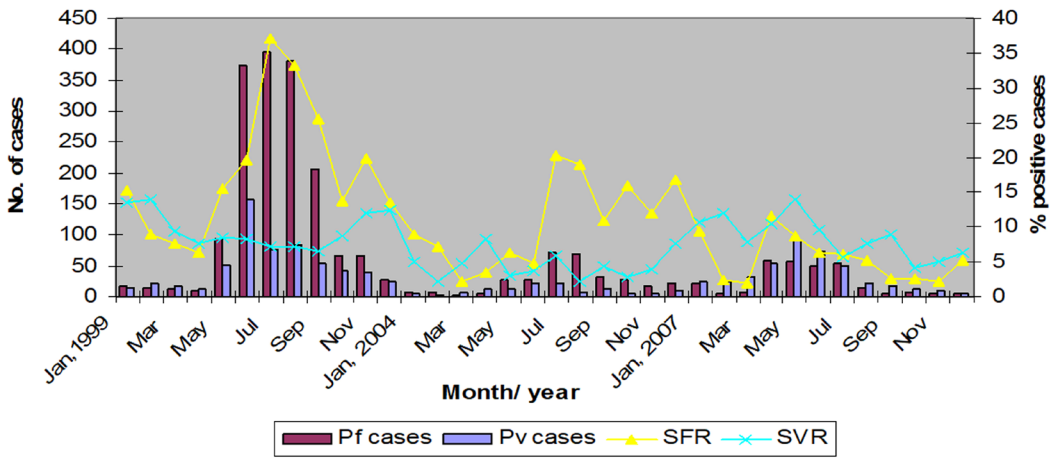


Figure 7. Declining trend of malaria transmission in the Sonapur Primary Health Centre (Dimoria block) of Kamrup district, Assam consequent to change in drug treatment policy from chloroquine to sulfadoxine-pyrimethamine in 2004, and to ACT (artesunate+ sulfadoxine-pyrimethamine) in 2007 (representative year 1999 when chloroquine was in force for treatment of *Plasmodium falciparum*). Pv = *Plasmodium vivax*, Pf = *Plasmodium falciparum*, SFR = % smear positive for *P. falciparum*, SVR = % smear positive for *P. vivax*. Source Reference [10]

There is a renewed hope and optimism with increased awareness on disease prevention and control helping restore confidence in communities at stake. Yet there is need to stay step ahead to overcome drug-resistant malaria for which monitoring should be the guiding principle placing improved drug-policy for radical cure. Entomological monitoring should be the continuing activity to raise alert on rising density of vector populations helping institute interventions well in time. These is no room for laxity at any stage of operation which can prove to be costly affair in reversing the gains. Instead, continued political commitment for increased allocation of resources for NE India is warranted for universal coverage of interventions and building equitable healthcare services in populations at any risk [25,26].

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8

Malaria transmission and containment practices in Tea Estates of Assam, North-East India

Introduction

In Assam, tea is the major industry and economic backbone of the state for its production (second largest producer in the world after China) providing direct employment close to a million in the state. It is a major cash crop and export commodity with over 800 Tea Estates (TEs) comprising small ($\leq$200 acres), medium (~400 acres) and large sized (>400 acres) spread on either side of Brahmaputra River and those in the Barak Valley. It is a rain dependent crop for which this region is advantageous for its tropical climate and high precipitation during April – September corresponding to malaria transmission season. Tea sector is a labour-intensive industry; an average sized tea garden provide employment to 4000 odd workers plus a few executives including managerial staffs, a medical doctor and paramedics providing primary healthcare; group of company gardens, instead have a central hospital for secondary level care of referral cases. Majority of work force are permanent labourers commonly referred as 'Tea Tribes'(~6.5 million constituting 18% of Assam's total population) initially brought by the British colonial planters as indentured labourers from the regions of the present-day Jharkhand, Odisha, Chhattisgarh, West Bengal and Andhra Pradesh into colonial Assam during 1860-1890s in multiple phases for the purpose of being employed in the tea gardens. Most labourers are provided quarters grouped in labour lines (formerly known as coolie lines) in the garden premises itself. Besides, there are temporary seasonal workers contracted locally during peak harvesting season hailing from nearby villages/hamlets located in the reserve forest areas often termed as 'Adivasi' (indigenous ethnic tribes). In the pre-DDT era before independence, many of the TEs suffered huge economic losses due to ravages of malaria depleting manpower on account of sickness/absenteeism resulting in low productivity [1,2]. Malaria was common ailment and sufferings were enormous with abundance of *Plasmodium falciparum*, *P. vivax* as well as *P. malariae* cases. For malaria containment, research investigations in TEs (controlled population with minimal migration) yielded valued information in understanding disease transmission and biology of mosquito vector species helping attempt naturalistic control interventions [3-5]. *Anopheles minimus* was incriminated by detection of sporozoite infections and a great deal of information was generated on its seasonal abundance and breeding characteristics held as major vector in the Assam valley [6-11]. With the advent of DDT during post World War II, naturalistic measures were overweighed by efficacy and long residual effect of insecticides, large-scale applications of which resulted in steadily decline of cases [12-16]. It was during 1970s that malaria had resurged with vengeance and disease outbreaks were common sight across the Assam valley [17,18]. Many of the TEs particularly those located along north bank of Brahmaputra River sharing border with Bhutan and Arunachal Pradesh were adversely affected reporting high rise in malaria cases and attributable deaths [19-23]. To help formulate appropriate intervention strategies, malaria surveys were undertaken in some of these tea estates in liaison with Assam Branch Indian Tea Association (ABITA) to submit specific recommendations for effective management. Included in this report are

salient research findings on disease vectors and prevalence of malaria for benefit of tea industry to check its spread.

Zoogeography and demographic information (Figure 1,2)

Tea plant (*Camellia sinensis*) is an evergreen bush and grows naturally in Assam and West Bengal. In Assam, most of the TEs are generally located on the outskirts of the township area often in juxtaposition to reserve forest along interstate/international borders. Tea garden communities cut off from the mainstream and recluse away from city life have evolved their own cultural identity. Most of these are illiterate living in low socio-economic conditions and prone to poor health due to endemic diseases and ailments, viz., malaria, tuberculosis, malnutrition, night-blindness, anaemia, and the like [24].

Figure 1. Top: Lush green Tea Estates sprawled over acres are common sight in Central and Upper Assam districts. Tall trees in between provide shelter to tea bushes against scorching heat in peak harvesting season. Bottom:Tea tribes, the permanent labour force engaged in harvesting and plantation are supported by garden management providing rent-free accommodation,access to healthcare services,subsidized ration and liveries. Besides basic wages per day their family income is supplemented by additional amount for quantity of leaves plucked in excess to required minimum by weight.

Tea tribes are unique in many ways characterized by costumes, lifestyle, food preferences and rituals having little interactions with local Assamese populations. Majority of TEs located in the central and upper Assam districts on north bank of Brahmaputra River

were malaria ridden reporting focal outbreaks characterized by sudden spurt of cases and attributable deaths. The landscape is studded with plenty of criss-crossing perennial seepage water streams, which originate from surrounding hills and passage through garden premises, are species-specific breeding habitat of *An. minimus*, the mosquito vector species [25]. Reserve forests (rich in wildlife) are depleting unchecked at the expense of deforestation by unauthorized human settlements/hamlets and increased acreage under cultivation [26]. Many of these hamlets (often located on banks of streams for source of water) are deprived of healthcare services least the interventions against malaria and other vector-borne diseases inter-alia do not seek treatment for lack of awareness. Consequently, good number of temporary labour force/seasonal tea garden workers living in these hamlets are rendered vulnerable to malaria attacks and extended morbidity.

Figure 2. Top: Any time is teatime, the favoured pastime of Britishers and million others; left - two leaves and a bud constitute the premium quality tea; right - tea leaves are regularly harvested by garden labourers for which they hold adequate skill for doing over generations; Assam is the second largest producer and exporter of CTC (crush, tear, curl) tea globally. Bottom: left - a panoramic view of labour line located within garden premises providing free living quarters to permanent labour force; right - a typical household family having free access to basic amenities and healthcare facility; quarters mostly made of split bamboos with asbestos/tina roofing are ideal resting sites of mosquito vector species located within reach <1 km of streams (breeding habitat) trespassing the garden premises.

Deforestation is resulting in altered ecology affecting fauna and flora/ecological succession. While populations of some mosquito species are depleting [27,28], newer vector species, *An. culicifacies* (formerly occurring in low densities) is establishing foot hold in the uncharted territories opening new vistas making malaria control a difficult proposition being multi-resistant to wide array of insecticides [29-33]. Several permanent tea garden labourers' own plots of land in these hamlets to supplement their income for which cross-migration is unstoppable. Herds of elephants often traverse through tea garden premises and hamlets creating niche for yet another efficient mosquito vector, *An. baimaii* (member

species of the *An. dirus* species complex) that are recorded breeding in rain water collections in forests pools and elephant footprints alike [28]. Both *An. minimus* and *An. baimaii* are proven efficient vectors of malaria; together resulting in cluster of cases often more than one death in the same household/locality [34]. Inhabitants of these hamlets dwelling on meagre wages/forest resources are largely ignorant and face the wrath of malaria and allied sicknesses perpetuating poverty amidst penury.

Malaria transmission: morbidity and mortality

Assam is malaria endemic and tea estates contribute substantial number of cases annually [35]. For data based on Tarajulie Tea Estate, it was evident that transmission is perennial with seasonal peak corresponding to wet season (April – September) coinciding with the harvesting period (Table 1). During this period, increased attendance in outpatient clinic was largely due to malarial fever across tea garden hospitals. Of the two prevalent causative parasite species, *P. falciparum* (the killer parasite) was invariably the majority parasite, the remaining were due to *P. vivax*. Among other plasmodial species, *P. malariae* infections, which apparently were prevalent in pre-DDT era [1,2], had become scarce or possibly disappeared [36]. Cases were recorded in all age groups, nevertheless, distribution of cases was heterogeneous (Table 2). While malaria prevalence in infants (<1 year age group) was strong evidence of ongoing active transmission, morbidity was manifold in >15 years age group. Most malaria-attributable deaths were also recorded in this age group and were ascribed to late reporting and consequent delayed treatment [21].

Table 1. Meteorological data and seasonal transmission of malaria in Tarajulie Tea Estate (Tezpur Circle), Assam, North-East India*

Month, 1991	Mean Temperatures		Rain-fall (mm)	Passive case detection (outpatient malaria clinic)				Malaria - attrib-utable deaths
	Mini-mum (^{0}C)	Maxi-mum (^{0}C)		No. of fever/ clinical cases screened for ma-laria parasite	No. blood-smears +ve for malaria parasite	Parasite rate (%)	% *Plasmodium falciparum* cases	
Jan	9.0	22.5	17.4	46	0	0	0	0
Feb	13.5	25.5	34.6	35	1	2.9	100	0
March	17.5	29.0	52.8	97	7	7.2	0	2
April	19.5	28.0	113.6	246	34	13.8	26	1
May	22.0	27.0	473.2	513	122	23.8	34	2
June	25.5	30.0	574.2	886	246	27.8	58	2
July	26.5	32.5	329.4	168	83	49.4	34	3
August	26.0	31.5	383.4	206	52	25.2	42	2
Sept	25.0	30.5	479.2	103	17	16.5	59	0
Oct	22.0	29.5	244.2	92	13	14.1	31	1
Nov	14.0	27.0	13.2	29	8	27.6	37	1
Dec	9.5	22.5	23.0	24	6	25.0	0	0

*Source: Tarajulie Tea Estate Hospital for data based on 1991

Table 2. Passive case detection in Tarajulie Tea Estate (Tezpur Circle) and adjoining hamlets, Assam, North-East India*

Study site (population)	Age group years	No. of fever cases screened for malaria parasite	No. & (%) blood-smears +ve for malaria	
			Plasmodium falciparum	*Plasmodium vivax*
Tarajulie TE (3781)	<1	94	16	3
	1 – <5	292	61	8
	5 – 15	206	64	13
	>15	403	115	42
	Total	**995**	**256 (25.7)**	**66 (6.6)**
Tengabil Hamlet (838)	<1	0	0	0
	1 – <5	3	3	0
	5 – 15	3	3	0
	>15	7	5	0
	Total	**13**	**11 (84.6)**	**0**
Kalabil Hamlet (1379)	<1	1	1	0
	1 – <5	5	1	0
	5 – 15	5	2	3
	>15	20	14	3
	Total	**31**	**18 (58)**	**6 (19)**
Lutera Hamlet (961)	<1	4	1	1
	1 – <5	10	3	0
	5 – 15	7	3	0
	>15	11	5	4
	Total	**32**	**12 (37.5)**	**5 (15.6)**

*Study period: May – June 1992

Malarial outbreaks were of common occurrence in TEs located in north bank associated with high rise in cases and attributable deaths (Figure 3). Malaria surveys in these TEs revealed that malaria was rampant and by far the major public health illness affecting industrial productivity adversely (Table 3). It evolved that whereas *P. falciparum* was the predominant infection in TEs located on north bank of Brahmaputra River (>60%); conversely *P. vivax* constituted the majority infection on south bank [20]. Parasite rate, however, was much higher in TEs of Mangaldai, Barsola and Lakhimpur circles sharing border with Bhutan and Arunachal Pradesh to the north (>40%), while in Dhubri, Tezpur, Biswanath, Nagaon, Jorhat, Doom Dooma and Margherita circles of TEs, parasite rates were <40% of fever cases examined.

Mosquito fauna and vector bionomics

Entomological investigation in TEs reporting focal disease outbreaks revealed that of more than 20 anopheline species reported in Assam [37]; *An. vagus*, *An. annularis*, *An. minimus*, *An. culicifacies*, *An. varuna*, *An. philippinensis/nivipes*, *An. splendidus*, *An. aconitus* and *An. jeyporiensis* were most abundant occurring in varying proportions [19]. Among these, *An. culicifacies* mosquitoes were recorded to prevalent in good numbers in Sonitpur district (Central Assam) otherwise occurring in low densities in districts of lower Assam. There are reports of faunistic changes in favour of building densities and incrimination of *An. culicifacies* in districts of Central Assam attributed to deforestation [29-33]. Nevertheless, among total mosquito collected, *An. minimus* mosquitoes were captured in good numbers in whole-night human-landing catches and were incriminated by detection of motile sporozoites in salivary glands; all other mosquito species dissected were sporozoite negative (Table 4). Among others, *An. minimus* mosquitoes had strong predilection for human host and actively foraged for blood-meal during the night with peak biting activity occurring between 21-22 till 3-4 hrs/day-break (Table 5). Thus, *An. minimus* was re-incriminated as major vector transmitting malaria in TEs investigated unequivocally. Both *An. minimus* and *An. culicifacies* were recorded breeding in slow-flowing seepage water streams in forest hamlets.

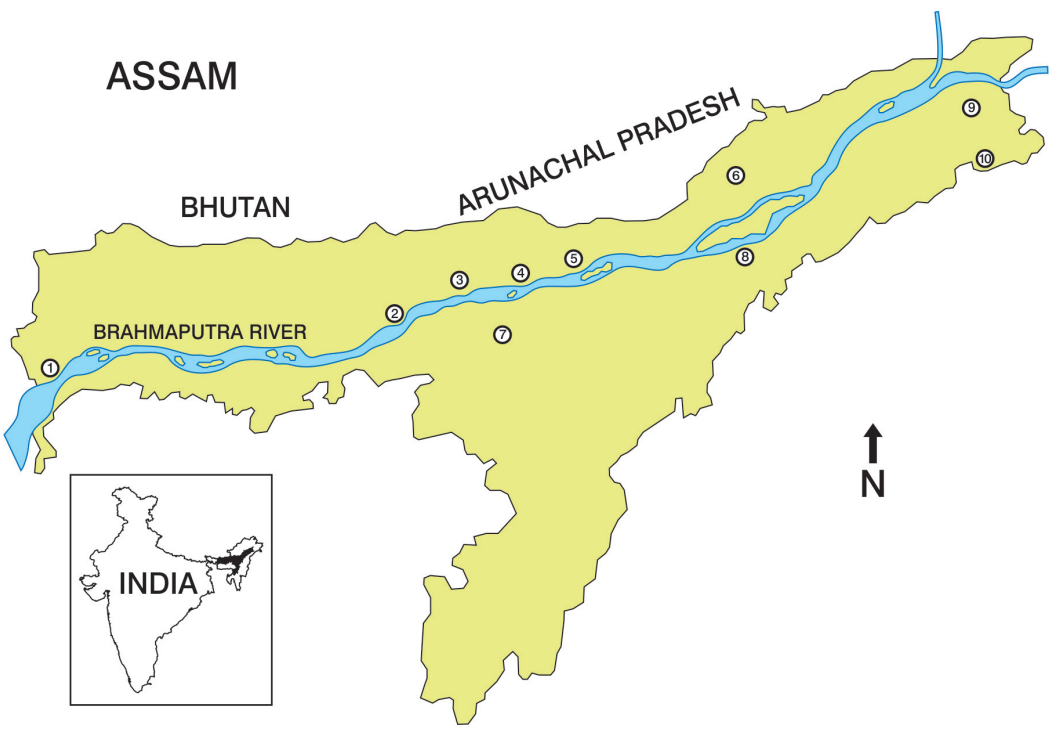

Figure 3. Map of Assam showing geographical location of various circles of Tea Estates investigated under Assam Branch Indian Tea Association (ABITA). The consecutive number 1-10 correspond to Dhubri, Mangaldai, Borsola, Tezpur, Biswanath, Lakhimpur, Nagaon, Jorhat, Doom Dooma and Margherita circle respectively. The inset is the map of India showing location of Assam, in the North-East region of India. Source Reference [20]

Table 3. Prevalence of malaria based on passive case detection (outpatient malaria clinic) in Tea Estates of Assam, North-East India*

S. No.	Circle	Tea Estate	Study period	No. of fever cases screened for malaria parasite	No. blood-smears +ve for malaria	Parasite rate (%)	% smears +ve for *Plasmodium falciparum*
1.	Dhubri	Choibari	Sept. – Oct., 1994	1000	390	39	69
2.	Mangaldai	Paneery	July – Aug., 1992	2953	1657	56	84
			April – June, 1993	1090	516	47	73
			May – June, 1994	504	307	61	82
		Bhootiachang	July – Aug., 1992	639	222	35	66
		Barangajulie	July – Aug., 1992	685	303	44	78
		Corramore	Dec. 92 – Jan. 1993	683	293	43	48
		Dimakuchi	July – Aug., 1992	70	29	41	76
		Attarikhat	July – Aug., 1992	355	194	55	84
3.	Borsola	Bettybari	July – Aug., 1994	1206	520	43	55
		Bahipukhuri	Aug. – Sept., 1994	834	416	50	71
4.	Tezpur	Tarajulie	May – June 1992	1071	374	35	77
		Kolony	May – June 1993	417	50	12	78
		Sonajulie	May – June 1993	121	19	16	79
		Naharoni	May – June 1993	14	7	50	86
5.	Biswanath	Majuligarh	Aug. – Sept., 1993	1129	249	22	92
		Monabari	Aug. – Sept., 1993	11	1	9	100
		Gingia	Aug. – Sept., 1993	23	3	13	100
		Pabhoi	Aug. – Sept., 1993	24	8	33	87
		Pratapgarh	Aug. – Sept., 1993	12	5	42	80
6.	Lakhimpur	Koilamari	June – July 1994	1439	679	47	85
7.	Nagaon	Kondoli	Oct. – Nov., 1993	425	99	23	33
8.	Jorhat	Diffloo	May – June 1994	567	164	29	21
		Methoni	May – June 1994	49	19	39	31
		Naharjan	May – June 1994	51	14	27	21
		Behora	May – June 1994	109	39	36	23
9.	Doom Dooma	Duamara	Sept., 1994	703	93	13	75
10.	Margherita	Margherita	May – June 1994	73	2	3	100

*Source Reference [20]

Table 4. Mosquito vector abundance and incrimination data in Tarajulie Tea Estate (Tezpur Circle) and adjoining hamlets, Assam, North-East India*

Anopheles mosquito species	Tarajulie TE	Adjoining Hamlets	Vector incrimination		Parity			
	Mosquito density per person hour		Total dissected	No. gland positives (%)	Total dissected	NP	1P	2P	% Parous
An. culicifacies	0.33	1.40	68	0	35	10	18	7	71.4
An. minimus	0.00	2.68	142	6 (4.23)	90	43	40	7	52.2

*Study period: May – June 1992. NP denotes non-parous, 1P = uniparous and likewise

Table 5. Mosquito biting behaviour for data based on whole night human landing catches in Paneery Tea Estate (Mangaldai Circle), Assam, North-East India*

Anopheles mosquito species	No. mosquitoes collected during the hour of											Total	Mean landing rate per person/ night* (Person nights 5)
	18-19	19-20	20-21	21-22	22-23	23-24	0 - 1	1 - 2	2 - 3	3 - 4	4 - 5		
An. annularis	0	3	1	2	1	1	1	1	0	0	1	11	2.0
An. fluviatilis	0	0	1	0	0	2	1	0	0	1	0	5	1.0
An. minimus	6	7	8	11	13	12	4	10	13	11	3	98	19.6
An. nivipes	1	1	0	0	0	0	0	0	0	0	0	2	0.4
An. splendidus	1	1	1	0	1	0	0	1	0	0	0	5	1.0
An. varuna	0	2	1	2	2	2	1	1	4	2	1	18	3.6

*Study period: July – August 1992

Risk factors and containment practices

Most of the TEs in Assam are managed by corporate sector providing health care services inclusive of interventions against malaria and other mosquito vector-borne diseases gratis, these included DDT spraying operations indoor labour quarters and passive case detection and treatment as per state protocols. Nevertheless, these services were extended only to permanent labour force residing within garden premises. The population groups residing in adjoining villages/hamlets located in reserve forest are unauthorized human settlements, thus neither served by garden authorities nor by the state and left unattended for all purposes and remained at much greater risk of contracting malaria. Yet, good number of temporary garden workers hailing from these hamlets work on daily basis in TEs for livelihood. Besides, many permanent residents of TEs own plot of land in hamlets for agriculture purposes to supplement family income and frequently halt for the night in peak transmission season coinciding with paddy cultivation/malaria transmission, thus exposed to infective mosquito bites. During malaria outbreak investigations, it was observed that malaria parasite rate was much higher in adjoining hamlets than garden labour lines, most of which were *P. falciparum* cases (Table 6). Besides, while *An. minimus* (the vector mosquitoes), which were not recorded to occur in labour lines located within the garden premises; these were widely prevalent in adjoining hamlets and incriminated (Table 4). Consequently, there was large concentration of cases in the hamlets for yielding more than one case/death per household located in closer proximity (<1 km) to perennial seepage water streams (the breeding habitat of *An. minimus*) for high degree of anthropophagy and low flight range (~1 km). Malaria cases detected in garden labour lines were unequivocally attributed to migration (to and fro) of garden labour force from (intervention area) to adjoining hamlets (non-intervention area) for having admitted owning a plot of land and staying overnight, apparently the site of acquisition of infective mosquito bites (Table 7). Thus, it was held that inter-alia: (i) cross-migration between garden premises to adjoining hamlets and, (ii) making night halts (*An. minimus* is night biting mosquito species) in non-intervention areas were the two major risk factors requiring attention of programme and policy managers to contain malaria for remedial actions specific to tea gardens.

Table 6. Spatial risk of malaria for data based on mass blood surveys in Tarajulie Tea Estate (Tezpur Circle) and adjoining hamlets, Assam, North-East India*

Location (population)	Febrile			Afebrile		
	No. of fever cases examined for malaria parasite	No. blood - smears +ve for malaria (parasite rate)	No. *Plasmodium falciparum* cases of total +ve cases	No. of blood - smears examined	No. blood - smears +ve for malaria (parasite rate)	No. *Plasmodium falciparum* cases of total +ve cases
Labour line 1 (936)	9	7 (78)	7	291	32 (11)	31
Labour line 2 (868)	0	0	0	174	30 (17)	28
Labour line 4 (103)	0	0	0	51	1 (2)	0
Labour line 6 (665)	1	0	0	192	37 (19)	37
Labour line 7 (749)	9	8 (89)	7	150	14 (9)	14
Labour line 8 (212)	3	1 (33)	1	105	17 (16)	17
Tengabil Hamlet (838)	5	3 (60)	3	94	26 (28)	26
Kalabil Hamlet (1379)	6	5 (83)	5	84	33 (39)	33
Lutera Hamlet (961)	5	2 (40)	2	180	33 (18)	33
Total	**38**	**26 (68)**	**25**	**1321**	**223 (17)**	**219**

*Study period: May – June 1992

Table 7. Malaria transmission in Tea Estate was established due to labour migration between garden premises and adjoining hamlets located in reserve forest for data based in Tarajulie Tea Estate (Tezpur Circle), Assam, North-East India*

Parameters	Labour line number					
	1	2	4	6	7	8
Number of labour quarters surveyed	34	43	11	31	9	26
No. of households having plot of land in hamlets	19	18	4	14	6	13
No. of families visiting hamlets						
Daily	6	5	1	8	3	5
Weekly	3	4	2	2	3	2
Monthly	4	5	1	1	0	0
Seasonally	6	1	0	3	0	3
Migration pattern						
Entire family	3	6	2	7	2	0
Parents only	3	3	1	1	2	5
Individual	13	6	1	6	2	5
% Households using mosquito nets	59	53	82	97	66	53
% DDT spray coverage	70	100	91	100	77	93
No. of families halting for the night in hamlets	8	5	1	11	3	5

*Source Reference [19]

Conclusions, and specific recommendations

Tea gardens have obvious advantage for research investigations being closed community for most populous living in the premises making follow up lot easier than said and done. It was apparent that TEs are malaria-endemic and were prone to focal disease outbreaks affecting productivity. While tea estates are well guarded and provided due healthcare services to labour force residing within premises, the problem had arisen from the adjoining hamlets/villages in reserve forests inhabitants of which are devoid of access to interventions having little awareness on disease prevention and treatment [38]. These population groups were left unattended years together resulting in pool of asymptomatic reservoir that served as continuous source of infection to the visitors evidenced by high parasite rate in afebrile groups. Apparently, hamlets were the site of acquisition of infection during night halts corroborated by entomological data for vector prevalence and infectivity. Vector mosquitoes, *An. minimus* were recorded and incriminated only in hamlets (non-intervention areas) vis-à-vis tea garden premises (intervention areas). Cross-migration of garden labour force residing in labour lines located within garden premises to the adjoining hamlets was the root cause evidenced by frequent visits except labour line No. 4 (the garden protection force having no migration history) in which cases were few and far; rest all labour lines were reporting cases. Based on these empirical data the following recommendations were reached: (i) to extend the interventions against malaria beyond garden premises at least up to one km radius (the flight range of vector mosquito), investment thus made would help reducing case load and absenteeism to maintain productivity; (ii) besides ensuring early case detection and treatment to prevent spread of malaria, prevention should also be strengthened by provision of mosquito nets (preferably long-lasting insecticidal nets) coupled with community awareness campaigns for compliance; (iii) above all, upgrading skills of medics as well as paramedics should be the continuing activity to keep abreast with the latest technologies in diagnostics and treatment. All these recommendations were subsequently accepted and implemented by the garden management resulting in appreciable transmission reduction; most TEs are presently relatively malaria-free barring few sporadic cases (Source: Indian Tea Association, personal communication). Nevertheless, there is a dire need to make continued adequate provision of resources providing services uninterrupted and maintaining vigil before malaria resurfaces again.

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9

Landscape epidemiology: disease transmission and risk factors of malaria

Introduction

North-East (NE) India comprising seven sister states namely Arunachal Pradesh, Assam, Manipur, Mizoram, Nagaland, and Tripura is the eastern most region of India landlocked by international border with Tibet Autonomous region of China in the north, Myanmar to the east, Bangladesh to the South and Bhutan to the west. NE India occupies 8% of total land area of India and is home to many indigenous tribes with large tracts of evergreen rainforest reserve supporting diverse fauna and flora. It is a vast geographical stretch including mountainous areas (montane climate), undulating hills and valleys marked by major river systems (sub-tropical climate). Large parts of NE India receive heavy rainfall (2-3 meters) during April - September mostly under the influence of southwest monsoon. All seven sister states, collectively sharing 4% of the Indian population, are malaria endemic contributing nearly 10-12% of reported cases (90% of which are *Plasmodium falciparum*) and 20% of malaria-attributable deaths in the country [1]. Historically, malaria is by far the major public health illness spread across the valleys [2]; the disease transmission and distribution of cases, however, is heterogenous with varied risk factors [3]. Malaria transmission is typically rural supported by *Anopheles minimus* and *An. baimaii*, the two major vector species in the region that cut across forest/tribal/border malaria ecotypes [4]. Lot many malaria cases remain undetected/unreported and left untreated due to poor surveillance and lack of awareness on disease prevention and control. Morbidity is enormous and access to health services is largely concentrated in town areas providing secondary/tertiary care [5]. Large segments of population groups, particularly those living in forest-fringes are at greater risk of infection and prone to disease outbreaks. To contain the disease onslaught, it is imperative to delimit high-risk areas helping prioritize resources to ensure equitable access and disrupt malaria transmission. The focus of this chapter is to delineate risk-factors down to the household level which will be helpful in implementing interventions in space and time to reduce morbidity and saving lives.

Distribution of malaria in relation to landscape, age-group and gender

Among NE states, Assam sharing 66% of total population of NE India, is the most populous and encompasses plain valleys and foothills traversed by major river systems and tributaries supporting livelihood of the masses. All other sister states are mostly hilly having stretches of foothill plains receptive for malaria transmission. Paddy cultivation is the major agricultural produce for subsistence grown at risk of malaria for providing aquatic habitat for proliferation of mosquito species having implication in malaria transmission. Assam is endemic for both *P. falciparum* and *P. vivax* malaria, distribution and relative prevalence, however, varies among reporting districts [6,7]. Point prevalence

studies during 1991-2000 across high-risk districts of Assam revealed that malaria was of common occurrence, but transmission intensities varied among districts investigated (Table 1). Parasite rate among fever cases ranged any where from 11.8% in Tinsukia (Upper Assam) to 56.1% in Darrang district (Lower Assam). Most were *P. falciparum* cases (>60%) except in Nagaon and Golaghat (Central Assam) where *P. vivax* predominated. Malaria cases were recorded in all age groups of both sexes equally, however, *P. falciparum* cases were more abundant in (5–15) year age group (Table 2). Micro-stratification of data down to the village level revealed that the relative risk varied in relation to physiographic locales (Figure 1) [8]. There were far more cases in villages located nearer to the seepage water streams, the preferred breeding habitat of *An. minimus*, foothill villages and those located farther from the healthcare facility (Table 3). Villages/human settlements located $\leq$1 km to seepage water streams were at greater risk than those located farther from breeding habitat (relative risk = 10.46; P<0.0001). Similarly, people living in foothill villages (practicing shifting cultivation) had significantly higher API (number of cases per thousand population) than living in plain areas (relative risk = 3.63; P<0.0001). Further more, malaria incidence was consistently higher in villages located >5 km away from the nearest healthcare facility than those located in villages located < 5 km away (relative risk = 3; P = 0.0013). Final model of the multivariate analysis of risk factors, selected using backward elimination procedure, indicated that among the risk factors investigated, distance from the breeding habitats and geographic location of human settlements were the most significant in order of their importance (P<0.0001), and the risk due to distance from healthcare facility was confounded with the latter.

Table 1. Prevalence of malaria in rice-growing districts of Assam valley, North-East India (1991 - 2000)

District	Year	No. fever cases examined	No. +ve for malaria parasite	No. +ve for *Plasmodium falciparum*	Parasite rate (%)	% *Plasmodium falciparum*
Karbi Anglong	1991	606	183	154	30.19	82
Kokrajhar	1992	649	303	279	46.69	79
Sonitpur	1992	4826	2560	2066	53.04	81
Darrang	1992	2953	1657	1418	56.11	86
Nagaon	1993	425	99	33	23.29	33
Dhubri	1993	1000	390	268	39.00	69
Golaghat	1994	567	164	35	28.92	21
Tinsukia	1994	805	95	72	11.80	76
Lakhimpur	1994	1439	679	579	47.18	85
Goalpara	1995	2144	1026	739	47.85	72
Kamrup	1998	4004	1124	683	28.07	61
Morigaon	1999	630	274	248	43.49	90
Jorhat	1999	504	81	73	16.10	90
Nalbari	2000	1116	437	350	39.16	80

Table 2. Distribution of malaria cases by age and gender in the Sonapur Primary Health Centre, a typical foothill malaria-endemic of Kamrup district, Assam, North-East India

Age group in (years)	No. of villages	Population	Year	Sex	No. fever cases examined	No. +ve for malaria parasite	No. +ve for *Plasmodium falciparum*	Parasite rate (%)
<1	33	11546	1987	Male	53	13	10	24.53
				Female	63	19	14	30.16
	54	23031	1988	Male	84	23	17	27.38
				Female	81	25	14	30.86
	56	29537	1989	Male	116	26	20	22.41
				Female	110	26	19	23.64
1 - <5	33	11546	1987	Male	417	142	112	34.05
				Female	547	146	105	31.95
	54	23031	1988	Male	699	227	179	32.47
				Female	676	209	158	30.92
	56	29537	1989	Male	879	246	196	27.99
				Female	889	277	213	31.16
5 - 15	33	11546	1987	Male	848	273	216	32.19
				Female	777	256	196	32.95
	54	23031	1988	Male	1540	467	400	30.32
				Female	1250	423	358	33.84
	56	29537	1989	Male	1863	609	518	32.69
				Female	1874	556	473	29.67
>15	33	11546	1987	Male	1720	469	350	27.27
				Female	1539	361	285	23.46
	54	23031	1988	Male	3353	794	641	23.68
				Female	2439	548	481	22.47
	56	29537	1989	Male	4193	823	727	19.63
				Female	3353	706	580	19.87

Malaria transmission dynamics and distribution of cases in relation to zoogeography is similar to Assam in Arunachal Pradesh, Manipur, Mizoram, Meghalaya, Nagaland and Tripura, yet relative risk of infection was found to be much greater associated with distance (largely non-motorable) from the nearest healthcare facility in Arunachal Pradesh [9]. In these states, human population settlements are sparse and communication means are meagre often lacking public transport. Malaria transmission is typical of border/forest ecotype with hamlets located in forest-fringes along the interstate or international border (Figure 2). Invariably, these are also the areas with large concentration of indigenous tribal populations living in impoverished conditions dependent on forest produce. Nevertheless, parasite formula varied in relation to latitude with greater risk of *P. vivax* infection in northern NE states of Arunachal Pradesh and Nagaland (alpine climates) in comparison to southern states of Assam, Meghalaya, Mizoram and Tripura (warmer climates) in which *P. falciparum* predominated [3].

Figure 1. Malaria risk factors in Assam: Top, A typical house made up of split bamboos amidst paddy fields in the valleys, the preferred resting habitat for *Anopheles minimus*; Bottom, houses located in closer proximity to seepage-water streams (preferred breeding habitat for *An. minimus*), are at much greater risk of infection.

Seasonal abundance of parasite species and relative risk

Malaria transmission is perennial in large tracts of NE states except in areas situated at > 3000 ft above mean sea level elevation where reported cases are largely imported from down in the valleys. Cases were recorded during all months, nevertheless, there was marked rise in cases commencing rainy season in April up until its cessation in September/ October; for remainder of the season, transmission continued but intensity of transmission declined substantially (Table 4).

This seasonal pattern of prevalence of parasite species was consistent (Table 5). There were consistently far more *P. falciparum* cases during wet season (April-September) and

Table 3. Distribution of malaria cases in relation to risk-factors in the Sonapur Primary Healthcare Centre, a typical foothill malaria-endemic area of Kamrup district, Assam, North-East India. Source Reference [8]

Risk Factor	Population*	No. of fever cases**	Parasite rate (%)	Mean API***	Relative risk	95% confidence interval	p- value
Breeding habitat							
> 1 km	8057	2759	14.71	16.79	1****	-	-
<1 km	14458	23606	32.28	175.70	10.46	3.58 - 30.54	<. 0001
Location of human habitation							
Plain	12866	9106	23.68	55.88	1****	-	-
Foothills	9649	17259	34.01	202.78	3.63	1.85 - 7.12	<. 0001
Access to healthcare facility							
< 5 km	14742	11886	26.18	70.36	1****	-	
> 5 km	7773	14479	33.94	210.77	3	1.54 - 5.83	0.0013

* Data based on active fever surveillance in the given population category
** Total number of fever cases examined for malaria parasite during the years 1990 -1992
*** API denotes mean number of malaria cases per thousand of population per year
**** Reference category

Figure 2. Top: Human settlements in forest-fringe located in closer proximity to international border are at much greater risk of malaria. Bottom: A typical high-risk hamlet receptive for malaria.

parasite rate was significantly lower in dry season than wet season (relative risk = 0.71; 95% CI = 0.63-0.81; P<0.0001). Conversely, parasite rate of *P. vivax* was significantly higher in dry season than wet season (relative risk = 1.35; 95% CI = 1.26-1.45; P<0.0001). However, transmission intensities of both parasite species varied between years observed (P<0.0001).

Table 4. Monthly malaria parasite species composition in the Sonapur Primary Health Centre, a typical foothill malaria-endemic area of Kamrup district, Assam, North-East India*

Month (1988)	Rainfall (mm)	No. of fever cases Examined for malaria parasite	No. and (%) of blood smears		Monthly parasite incidence (no. of fever cases with parasitaemia/ 1000 population)
			+ve for *Plasmodium falciparum*	+ve for *Plasmodium vivax*	
January	11.00	1105	57 (5.2)	22 (1.9)	4.7
February	33.7	350	22 (6.3)	17 (4.8)	2.5
March	76.6	785	52 (6.6)	22 (2.8)	4.0
April	163.1	632	63 (10.0)	36 (5.7)	4.0
May	569.7	1066	225 (21.1)	46 (4.3)	11.8
June	322.1	1463	451 (30.8)	93 (6.4)	21.3
July	365.4	1755	293 (16.7)	53 (3.0)	13.5
August	329.9	1421	356 (25.0)	55 (3.9)	16.0
September	140.3	1530	288 (18.8)	40 (2.6)	12.3
October	71.7	1580	328 (20.8)	32 (2.0)	13.4
November	89.1	1206	206 (17.1)	46 (3.8)	9.4
December	16.3	956	132 (13.8)	58 (6.1)	7.0

*Data based on active fever surveillance in 50 villages (pop ~ 22,500)

Spatial and temporal abundance of mosquito vectors

Both the vector species, *An. minimus* (perennial species) and *An. baimaii* (monsoon species) were recorded in malaria-endemic districts of Assam and incriminated [10,11]. While *An. minimus* was widely abundant in the Assam valley throughout the year with peak densities corresponding to main transmission season (Table 6), *An. baimaii*, instead, had patchy distribution associated with forest cover/forest-fringe areas inlands as well as along the interstate and international borders (Figure 3); both having strong predilection for human host. Both these species have distinct bionomical characteristics (resting and breeding habitats) having implications in disease transmission and control options. Interestingly, formerly identified populations of *An. fluviatilis* have now been characterized to be seasonal variant of *An. minimus* down in valleys [12], nevertheless, true presence of *An. fluviatilis* cannot be ruled out with records of incrimination in higher hill ranges (1500m above msl) resulting in focal disease outbreaks even in winter months [13].

There are reports of population diminution of *An. minimus* and *An. baimaii* on account of altered ecology attributed to deforestation, human anthropogenic activities for increased

Table 5. Seasonal prevalence of malaria in the Sonapur Primary Health Centre, a typical foothill malaria-endemic area of Kamrup district, Assam, North-East India*

Year	Dry season (October – March)			Wet season (April – September)		
	No. of blood smears examined	No. &(%) of blood smears		No. of blood smears examined	No. &(%) of blood smears	
		+ve for *Plasmodium falciparum*	+ve for *Plasmodium vivax*		+ve for *Plasmodium falciparum*	+ve for *Plasmodium vivax*
1991	844	186 (22.0)	147 (17.4)	2301	702 (30.5)	262 (11.3)
1992	1376	397 (28.8)	170 (12.3)	3469	1063 (30.6)	309 (8.9)
1993	2090	531 (25.4)	275 (13.1)	3821	1079 (28.2)	379 (9.9)
1994	2219	400 (18.0)	320 (14.4)	6096	1746 (28.6)	656 (10.7)
1995	2249	334 (14.8)	415 (18.4)	7740	2002 (25.8)	964 (12.4)
1996	1887	236 (12.5)	343 (18.2)	4914	1014 (20.6)	686 (14.0)
1997	1144	127 (11.1)	156 (13.6)	3814	732 (19.1)	356 (9.3)
1998	1271	181 (14.2)	163 (12.8)	2723	502 (18.4)	278 (10.2)
1999	1446	204 (14.1)	160 (11.0)	5700	1467 (25.7)	422 (7.4)
2000	1213	137 (11.2)	136 (11.2)	2305	231 (10.0)	189 (8.1)
2001	1067	96 (8.9)	73 (6.8)	2822	467 (16.5)	146 (5.1)
2002	790	82 (10.3)	50 (6.3)	2648	293 (11.0)	197 (7.4)
2003	911	73 (8.0)	40 (4.4)	2043	163 (8.0)	112 (5.5)

*Data based on passive surveillance (malaria clinic). Source Reference [5]

acreage under cultivation, infrastructure expansion and the like [14, 15]. Consequently, populations of both these species are fast depleting and niche, thus, vacated is being accessed by *An. culicifacies*, the major vector in rural India [16]. Populations of *An. culicifacies*, formerly scarce are presently fast building in Assam and assessed to be multi-resistant to residual insecticides posing renewed threat to transmission control [17]. Besides these, there are several secondary vector species recorded breeding in rice-agroecosystem and have been implicated in transmission evidenced by sporadic records of seasonal sporozoite infections [18]. Among these, *An. philippinensis* and *An. nivipes* deserve specific mention. *An. philippinens* is and *An. nivipes*, the two closely related but distinct species, occur in varying proportions in NE states [19], however, their relative abundance, reported to vary in relation to elevation from sea level (Table 7). Of these two species, *An. nivipe*s was incriminated with seasonal infections for sporozoite antigen in ELISA assays.

High-risk areas and prioritizing interventions

Understanding landscape epidemiology holds merit in identification of the high-risk areas and prioritizing interventions to contain spread of malaria. The disease distribution is uneven and associated transmission risk varied in relation to topography and ecological determinants [7,8]. The relative transmission risk, particularly of *P. falciparum* malaria, is high in the NE states due to the climatic conditions and efficient vectors specific to the region. Seven sister states of NE India, sharing just 4% of country's population,

Table 6. Relative abundance of *Anopheles minimus* and infectivity in malaria-endemic districts of Assam, North-East India

District	Study location	Study period	Indoor resting collections (person hour density)	Whole night landing collections (per person per night)	Sporozoite infectivity rate (%)
Kamrup	Sonapur	June-Oct., 1988	7.00	14.00	3.31
Karbi Anglong	Manja	May 1991	7.00	-	2.84
Kokrajhar	Gossaigaon	July-Aug., 1991	2.00	-	3.37
Sonitpur	Rangapara	July-Sept., 1992	2.00	13.00	4.23
Darrang	Tangla	Aug.-Sept., 1992	11.00	20.00	3.14
Nagaon	Kathiatoli	Aug.- Nov., 1993	0.43	2.00	0
Dhubri	Chapar	Aug.- Nov., 1993	0	0	0
Golaghat	Bokakhat	May-July 1994	5.35	12.25	0.99
Lakhimpur	Lakhimpur	May-July 1994	0.09	0	0
Tinsukia	Margherita	May-July 1994	0	0	0
Goalpara	Agia	April-May 1995	0.95	18.75	2.86
Morigaon	Nellie	July-Aug., 1999	2.87	23.00	3.08
Jorhat	Titabor	May-June 1999	-	13.00	0
Nalbari	Kumarikata	June 2000	2.30	-	6.52

(-) denotes data not collected.

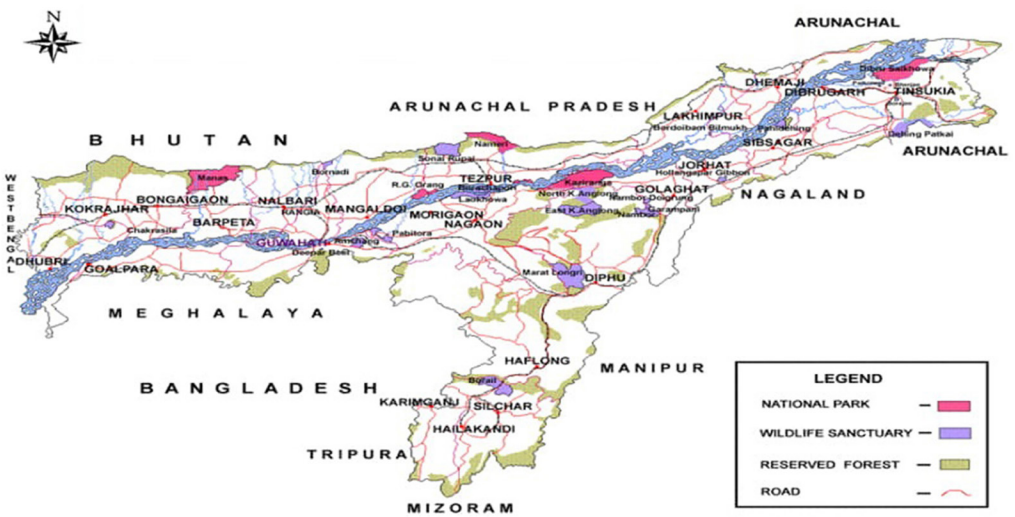

Figure 3. Map of Assam showing interstate and international borders and location of national parks, wildlife sanctuaries and reserve forests. Human settlements in forest-fringe areas along these borders are receptive for malaria transmitted by *Anopheles baimaii* (the forest dwelling mosquito species).

contributes 10-12% of malaria cases (90% of which are due to *P. falciparum*) and 20% of malaria-attributable deaths annually in the national scenario[2]. The location of breeding sources of vector species, forest cover, cross-border migration, housing conditions, access to healthcare services, distribution of indigenous tribal populations in juxtaposition to

forests/border areas, are all risk factors for active transmission of the causative parasites. NE India is the second largest biodiversity hotspot in the world and home to many native aborigines' concentrations of which varied across the states (Figure 4). These population groups living in impoverished conditions are at greater risk of infection for lacking awareness perpetuating poverty. Besides poor community awareness, subjects invariably do not seek treatment resulting in delayed reporting and fatal outcome [5]. Healthcare access in these areas is grossly inapt leaving many cases untreated resulting in pool of asymptomatic parasite reservoir and continued transmission. Disease surveillance can at best be described as fragmentary leaving populations vulnerable for lack of interventions years together. These are also the communities reporting fulminating disease outbreaks during which morbidity is insurmountable that often remain underreported. In this context, application of Geographical Information System/Remote Sensing Technologies holds promise but remain unexplored to delimit high-risk pockets helping target interventions averting impending disease outbreaks [20,21].

Table 7. Relative abundance of *Anopheles nivipes* and *Anopheles philippinensis* at different altitude ranges above mean sea level*

Anopheles Species	Number (%) of mosquitoes collected at different altitude ranges in metres (m)					
	0-199 m	200-399 m	400-599 m	600-799 m	800-999 m	1000-1300 m
An. nivipes	247 (60)	69 (17)	30 (7)	0 (0)	6 (1.5)	59 (14.4)
An. philippinensis	13 (13.4)	8 (8.2)	61 (63)	5 (5)	0 (0)	10 (10.3)

*Source Reference [19]

Conclusions

Landscape of NE states is rapidly transforming on account of expanding infrastructure of rail/road network, mushrooming industries, population explosion and urbanization; all at the expense of deforestation resulting in altered ecology and faunistic changes having implication in formulating intervention strategies [14,15]. While populations of *An. minimus* and *An. baimaii* are depleting [11,22], *An. culicifacies* is fast establishing in degraded forest areas [16,17]. The build-up populations of *An. culicifacies* complex of mosquito species would prove detrimental to control malaria for being multi-resistant virtually to all available insecticides [23]. Entomological data are critical to monitor vector densities and should be the cornerstone activity providing inputs to the control programme but remain poorly applied in addressing the programme needs [24]. To enhance capacities for vector surveillance, Spatial Decision Support System (SDSS) encompassing Geographical Information System (GIS) and Remote Sensing and allied technologies (Figure 5) should be incorporated to delimit high-risk areas helping prioritize interventions for proven utility in the field [25,26]. One-size-does-not-fit-all, thus, making right choice in right place, can yield rich dividends in reducing transmission risk for which changing landscape have direct bearing in formulating policy and planning for saving operational costs [27]. Evidence exits that intensification of interventions has resulted in paradigm shift in vector behaviour resulting in emergence of new ecotypes, viz., outdoor resting and biting requiring newer

Figure 4. Distribution of indigenous tribes in India. Percentages are cumulative for undivided states of Uttar Pradesh, Bihar, Madhya Pradesh and Andhra Pradesh. North-East India is markedly rich in ethnic tribes at risk of malaria requiring prioritization in resource allocation and strengthening interventions in the context of malaria elimination (Courtesy, V.P. Sharma)

tools to contain vector populations [28,29]. Drug-resistance (for which NE India is considered corridor to peninsular India) is fast evolving requiring regular monitoring of therapeutic efficacies for radical cure to prevent entry and spread of drug-resistant malaria [30].

To stay step ahead, before drug-resistant strains become widespread, disease surveillance should be intensified using all available modern tools, e.g., digital surveillance, helping micro-management down to the household level in the context of malaria elimination initiative [31]. As cases become scarce, correct diagnosis would become critical to rule out sub-microscopic/low-density parasitaemia which generally results in missing estimated

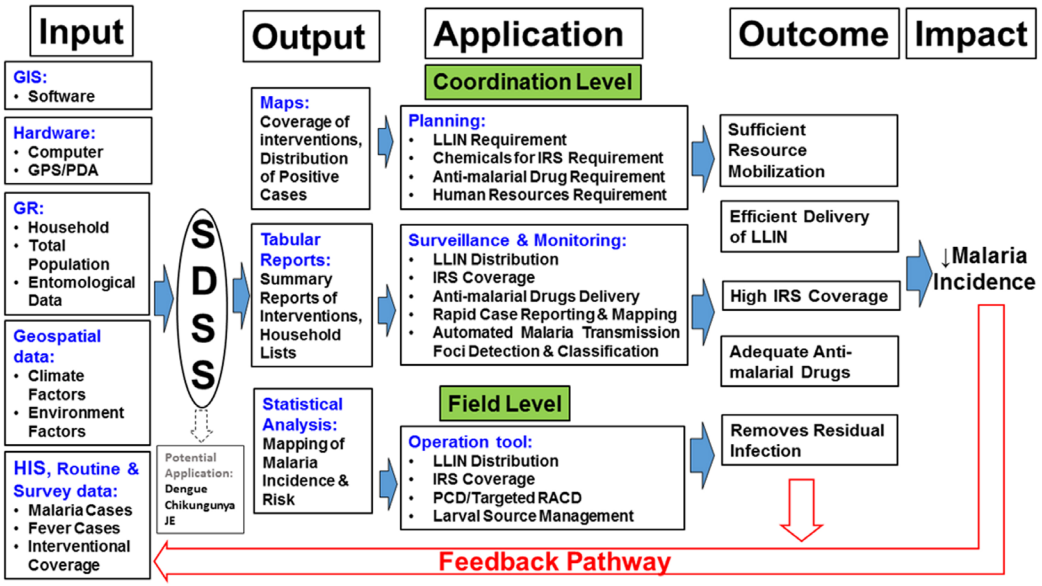

Figure 5. Framework of Spatial Decision Support System (SDSS) for malaria control and prevention with potential use in other vector borne diseases. (GIS: geographical information system; PDA: personnel digital assistant; GPS: Global positioning system; GR: geographic reconnaissance; LLIN: long-lasting insecticidal net; IRS: indoor residual spraying; PCD: passive case detection; RACD: active case detection; JE: Japanese Encephalitis).Source Reference [25]

one fourth of infections, thus, leaving these untreated [32]. Armed with newer diagnostics, there is imperative need to strengthen human resource and health delivery systems in out reaching population groups to disrupt transmission and emergence of drug-resistant malaria [33]. It is strongly advocated to allocate resources preferentially for NE India ensuring universal coverage of interventions prioritizing high-risk areas/hotspots which would go a long way in mitigating malaria onslaught and containing residual transmission [34]. Given the present-day intervention tools, disease transmission is steadily and surely declining across India for which NE India may become the forerunner in achieving malaria elimination by 2030 subject to continued political commitment for sustained allocation of resources [35].

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Section IV

Disease Surveillance and Drug-Resistant Malaria

10

Malaria surveillance: diagnostic techniques and emerging technologies

Introduction

Malaria surveillance is the key intervention providing species-specific treatment and has been the continuing activity ever since inception of the Indian national malaria control programme post-independence [1]. Laboratory services are essential components providing confirmed diagnosis to prevent mortality and reduce morbidity. Morbidity associated with *Plasmodium falciparum* (the predominant infection in the North-East India) is enormous and manifold than what is being reported due to irregular disease surveillance coupled with lack of community awareness and compliance. What has been considered more critical is the'Early Diagnosis and Prompt Treatment (EDPT)' to prevent complications associated with *P. falciparum* attack outcome of which is often fatal which alternatively could have been prevented [2]. Given the high receptivity for malaria in the North-East (NE) region, the case load remained insurmountable particularly in high transmission season resulting in unusual delays in administering radical cure [3]. The advent of rapid diagnostic test (RDT) kits'often termed as dipsticks' just about at turn of the century (2004-2005) was a big step forward helping strengthen surveillance enabling initiation of chemotherapy early at door-step [4]. A host of branded products for diagnosis of *P. falciparum* and combo-kits inclusive of *P. vivax* were made available for public utility. Before RDTs could be put into the programme, these were subject to field-evaluation in the given climatic epidemiological conditions for sensitivity and specificity helping formulate policy. Included in this communication is the comparative account of malaria diagnostic techniques inclusive of study results of available RDTs for data based on malaria-endemic block of Assam highlighting benefits and limitations, and role of some of the forthcoming technologies for confirmed diagnosis helping determine true incidence of malaria for benefit of the control programme.

Malaria microscopy: the gold standard

Among available techniques, diagnosis by microscopic examination of blood-smears (both thick and thin) continues to be in practice in the malaria control programme [5]. Even though procedure is labour intensive and requires some degree of technical expertise, it remains the gold standard technique for confirmation of parasite species. Malaria microscopy is the routine method in practice for identification of human *Plasmodium* species in the disease surveillance and remains indispensable for monitoring parasite density in therapeutic efficacy investigations of antimalarials. The indigenously developed Jaswant Singh and Bhattacharya (JSB) stain is commonly used for rapid staining providing equally good results compared to universally applied Giemsa staining procedures [6]. While thick smear helps quicker detection of malaria parasite, thin smear is screened for confirmation of

parasite species enabling dispensing appropriate therapy. Each human *plasmodium* species including *P. vivax*, *P. falciparum*, *P. malariae*, *P. ovale* has distinct morphology in the blood-stage infection. Among these, *P. falciparum* and *P. vivax* are widely abundant in North-East India and can be easily characterized, viz., while RBCs infected with *P. vivax* are often enlarged and have amorphous trophozoite coupled with Schüffner's dots; whereas RBCs infected with *P. falciparum* instead have characteristic ring-stage often with two chromatin dots and crescent shaped gametocyte (Figure 1).

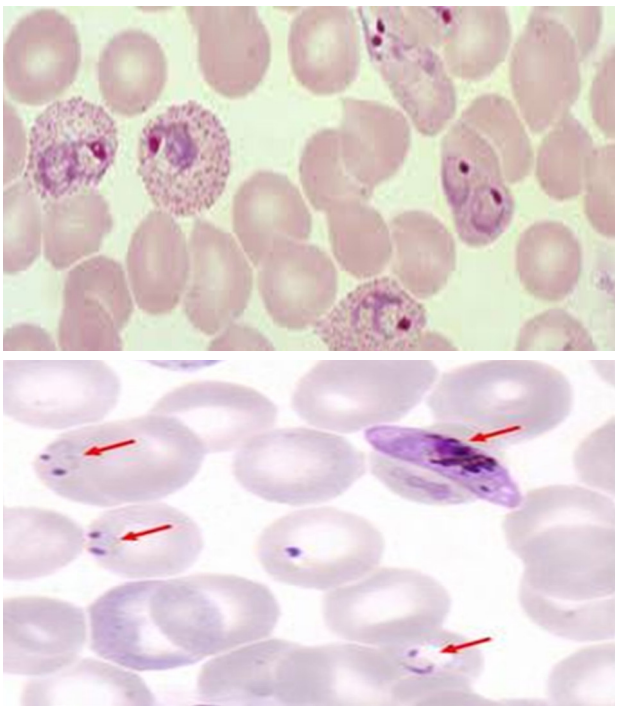

Figure 1. Thin blood film showing RBCs infected with human *Plasmodium* species; Upper: enlarged RBC with amorphous trophozoite characteristic of *Plasmodium vivax* with Schüffner's dots; Lower: RBC with characteristic ring-stage trophozoite and crescent shaped gametocyte of *Plasmodium falciparum*. Source: Wikipedia

However, even though malaria microscopy is cost-effective compared to molecular techniques, it has its own limitations including: (i) failing to detect low-density/sub-patent parasitaemia (<100 µl), (ii) requiring good quality smear and staining for accurate results (often poor preparation of smears and staining renders slide unreadable), (iii) difficult to practice in remote areas devoid of basic minimum requirements for laboratory set-up, (iv) requires considerable technical expertise for microscopic examination in reaching correct diagnosis [7]. Yet, malaria microscopy is fundamental to confirmed diagnosis and continues to be the core-activity of the programme for policy decision.

Quantitative Buffy Coat (QBC) method

In contrast to conventional microscopy, while Quantitative Buffy Coat (QBC) method offer advantages and held superior for greater sensitivity in confirmation of low-

density infections (<10 parasites per µl) providing much quicker results; the technique, however,often fails to discriminate parasite species what is critical to the programme for varied therapeutic regimens for radical cure [8-10]. The basic test principle of QBC involves binding of acridine orange with nucleic acids of parasite and the associated fluorescence is observable under blue-violet light through a microscope. The nuclei of the parasites emit yellowish green fluorescence whereas the cytoplasm exhibits bright red fluorescence. RBCs are not stained by the dye, hence remain inconspicuous under fluorescent light (dark background) while the brightly fluorescent parasites are easily seen. The outline of stained parasites is well preserved, and the general morphology is similar to that specimen stained by the Giemsa stain (Figure 2).

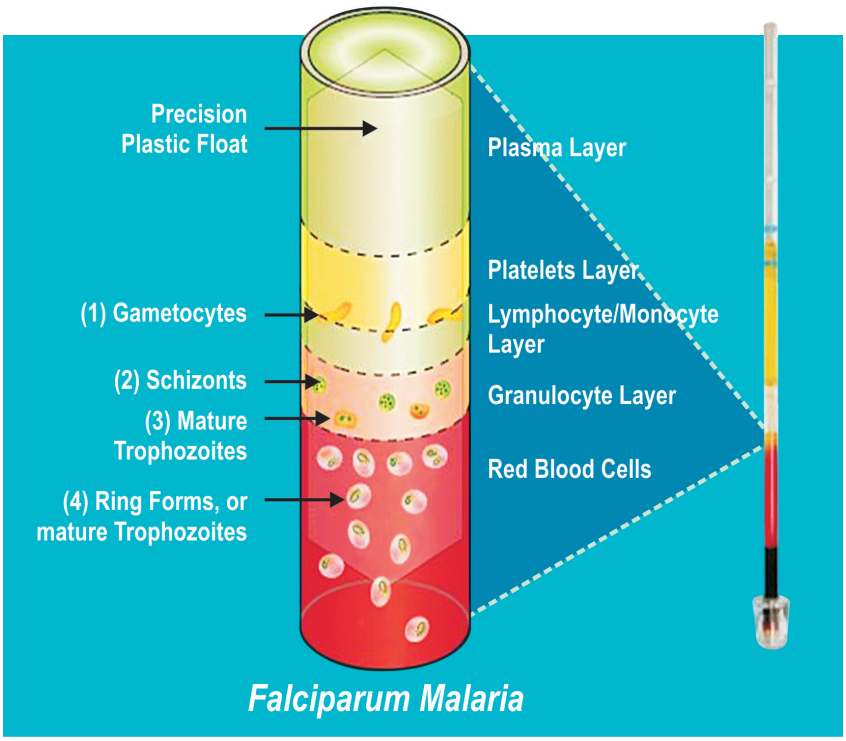

Figure 2. Quantitative Buffy Coad (QBC)test system: a diagrammatic representation outcome and interpretation. Source: Wikipedia

Partec rapid malaria test (PT) is very similar to QBC method requiring only test slide that is readily labelled with 4-6-diamidino-2 phenylindole (DAPI) which binds to intra-erythrocytic *Plasmodium* DNA resulting in fluorescence under Partec CyScope [11]. Both these methods of diagnosis are less laborious but require fluorescence microscope presently not included in the programme.

Rapid diagnostics test (RDT) kits: utility and limitations

While examination of thick and thin blood-smears as well as QBC methods require microscope and additional equipment; rapid diagnostic kits (RDTs) instead are easy to deploy and practicable in the field. The advent of RDTs is indeed a landmark development

providing on-the-spot result within 10-15 minutes of operation permitting initiation of treatment at the household level. Variety of branded products are made available and were subject to field-evaluation for test accuracy, sensitivity, and specificity in comparison to standard microscopic results by examination of thick and thin smears before these were operationalized. All these test kits are based on antigen-capture assay derived from malaria parasite in blood using immunochromatographic method and are easy to operate in the field conditions at the point-of-care with minimal skill having built-in control to validate the test results (Figure 3) [12]. The following three parasite antigens that have been exploited for development of RDTs enabling detection of malaria parasites in peripheral blood are enumerated as below:

Histidine-Rich Protein (HRP)

P. falciparum-infected RBC synthesize three histidine-rich proteins namely HRP-1 (the knob-associated HRP), HRP-2, and HRP-3. Among these, HRP-2 is the most abundant produced by asexual stages and young gametocytes of *P. falciparum*. It is a water-soluble surface protein expressed on the RBC membrane surface, and because of its abundance it was the first antigen used to develop an RDT for detection of *P. falciparum*.

Parasite lactate dehydrogenase (pLDH)

It is a soluble glycolytic enzyme expressed at high levels in asexual stages of malaria parasites. Different isomers of pLDH for each of the four Plasmodium species infecting humans exist, but their utility constitutes a second approach to RDT development. pLDH activity is well correlated with the level of parasitaemia in *in-vitro* cultures of malaria as well as in the plasma of infected patients determined by microscopy. pLDH based antigen detection kit is invariably used in conjunction with PfHRP-2 an integral component of combo-test kits for detection of non-falciparum malaria.

Aldolase

Aldolase is a key enzyme within the glycolytic pathway of the malaria parasite and has been considered as target antigen for rapid malaria diagnostic tests. Aldolase antigen-based kit is used exclusively for detection of *P. falciparum* and *P. vivax* antigens (not reactive to *P. malariae* and *P. ovale*), but its sensitivity is density dependent. However, unlike PfHRP-2 antigen-based kits, both aldolase and pLDH biomarkers are not linked to persistence of antigenemia.

Majority of available test kits are based on capture of circulating histidine rich protein-2 (PfHRP-2) antigen specific for *P. falciparum*. This test kit has been assessed to be highly specific with high degree of sensitivity (100%) and specificity (94-100%) in varied epidemiological conditions (Table 1) [13]. Even though rollout of these kits has helped ensure early diagnosis at point-of-care but not without limitations. One shortcoming associated with P*f*HRP-2 is the persistent antigenemia that continued to circulate days post-therapyup to 1-4 weeks even after clearance of parasitaemia having obvious implications (Table 2). This contrasts with pLDH and aldolase-based kits for persisting antigenemia lasting <10 days.

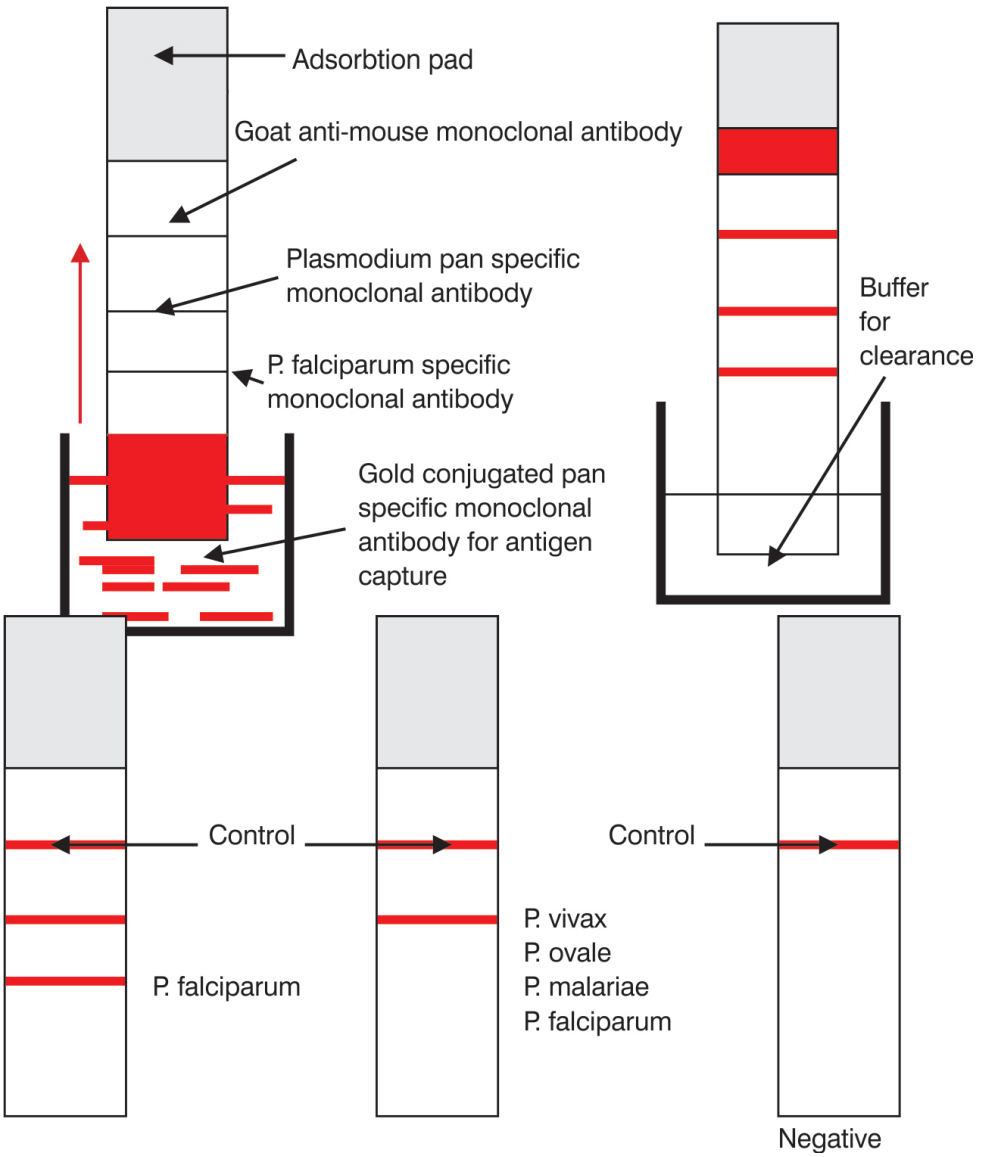

Figure 3. Diagrammatic representation of principle of immunochromatographic Rapid Diagnostic Test kit for diagnosis of malaria (see Box for test principle). Source Reference [12, reproduced with permission].

Test principle: The test is based on the principle of immunochromatography in which nitrocellulose membrane is coated with Anti-HRP-II/pLDH antibody (capture antibody) which is specific for *P. falciparum* and/or non-*falciparum* malaria. When the test sample along with Reaction Buffer flows through the conjugate pad, the colloidal gold coupled with Anti-HRP-II/pLDH antibodies (detection antibody) binds to HRP-II/pLDH antigens released from the lysed infected red blood cells of test sample. This antigen-detector antibody complex moves along the nitrocellulose membrane and binds to the corresponding immobilised antibodies to HRP-II/pLDH (capture antibody) leading to the formation of red colour band which indicates reactive results. The control band should appear irrespective of reactive or non-reactive sample which indicates successful migration of the reaction mixture/test validity.

Table 1. Field-evaluation of rapid diagnostic test kits for sensitivity, specificity and test efficiency for data based on typical malaria-endemic block of Assam, North-East India*

Rapid Diagnostic Test (manufacturer)	Microscopic results[a]			Test kit results			Sensitivity (%) TP/ (TP+FN)[f]	Specificity (%) TN/ (TN+FP)[g]	Test efficiency (%) (TP+TN)/ number of all tests
	Pf	Pv	Neg	Pf TP/FP	Pv TP/FP	Neg TN/FN			
ParaSight-F (Becton Dickinson, Cockeysville, MD, USA)[b]	25 (1)[e]	6	12	26/1	-	17/0	100	94	100
ICT Malaria Pf (ICT Diagnostics, Brookville, NSW, Australia)[b]	84 (1)	4	8	85/0	-	12/0	100	100	100
ParacheckPf (Orched Biomedical Systems, Goa, India)[b]	21	3	6	21/0	-	9/0	100	100	100
PataHit-f (Span Diagnostics Ltd., Surat, India)[b]	7	-	3	7/0	-	3/0	100	100	100
New Pf -1 mini (Monozyme India, Ltd, Secundrabad)[b]	6	2	2	6/0	-	4/0	100	100	100
SD Malaria Pf/Pv (SD Standard Diagnostics, Inc., Korea)[c]	17	7	6	17/0	5/0	Pf=6/0 Pv=6/2	Pf=100 Pv=71	Pf=100 Pv=100	Pf=100 Pv=85
DiaMedOptiMAL (Flow Inc., Portland, Oregon, USA)[d]	85	28	26	69/0	25/0	Pf=26/16 Pv=26/3	Pf=81 Pv=89	Pf=100 Pv=100	Pf=86 Pv=94

*Source Reference [14]

[a] Results based on microscopic examination of thick and thin smear.

[b] Diagnostic kits based on detection of free circulating HRP-2 antigen specific for *Plasmodium falciparum* (Pf).

[c] Diagnostic kits based on detection of free circulating HRP-2 antigen specific for *Plasmodium falciparum* and pan-malarial antigen for all human plasmodia.

[d] Diagnostic kits based on detection of parasite lactate dehydrogenase (pLDH) antigen specific for *Plasmodium falciparum* and pan-malarial antigen for all human plasmodia.

[e] Figures within brackets denote number of mixed infections (Pf+Pv) cases.

[f] TP and FN denote true positives and false negatives, respectively.

[g] TN and FP denote true negatives and false positives, respectively.

Consequently, diagnosis based on these test kits as that should be subject to confirmation by microscopic examination circumventing excessive use of antimalarials. Subsequently, combo-kits were made available incorporating pan-malarial monoclonal antibodies to detect circulating antigens of both *P. falciparum* as well as non-falciparum malaria parasite species (*P. vivax, P. malariae*, *P. ovale*). Among combo-kits, PfHRP2- and pLDH-based RDTs are the most commonly used; both these antigens are better option than aldolase-based kits for greater sensitivity.These combo-kits were ascertained to be sensitive and specific for *P. falciparum* (100%), but much less sensitive (~70%) for non-falciparum species (particularly at low parasite densities)of which while *P. vivax* is the most abundant; distribution of *P. malariae* is patchy and *P. ovale* is of rare occurrence in India [14]. While pLDH-based kits hold edge over PfHRP2 for added advantages, viz., (i) having potential in monitoring drug-efficacy investigations, (ii) lack of persistence antigenemia, (iii) lack of antigenic variations, (iv) prozone effect; yet the latter holds merit in detecting low-density parasitaemia including sequestered parasites and predicting progression of severe malaria [15].

The variation to test efficiencies of test-kits could be attributed to false negatives (perhaps due to non-viable parasites not producing LDH) and those declared positives might as well be due to sub-patent parasitaemia not detected by conventional microscopy [16,17]. Nevertheless, all these test kits are rather qualitative methods than quantitative having limited role in monitoring therapeutic response to treatments based on circulation of antigens coded by specific genes, deletions of which can amount to inaccuracies/false negative results. Naturally occurring deletions of histidine rich protein 2 and 3 (*Pfhrp2, Pfhrp3*) have been reported to be occurring in India as well as other malaria-endemic countries and perceived emerging threat to malaria elimination efforts [18,19]. Ever since, there has been substantial improvements in diagnostic sensitivity and specificity of RDTs aligning with PCR and microscopy gold standards and are widely used globally helping move away from the laboratory nearer to the patient [20]. Most present-day available bivalent RDTs incorporate antibodies against two or more antigens enabling to distinguish falciparum and non-falciparum malaria and are increasingly in demand universally providing easy-to-use low-cost sustainable intervention in resource-poor countries helping understand local disease epidemiology [21]. Bivalent RDT that is

Frequently Asked Questions on Rapid Diagnostic Test (RDT) Kits

Q. Can I use an RDT on more than one person?

Ans. No. Each cassette to be used only once. You need a new, unopened cassette for each patient. If you get an invalid result from one cassette, you need a new, unopened cassette to retest the same patient.

Q. Is it possible to get a positive result if the patient does not really have malaria?

Ans. Yes. If the patient has taken malaria medication in the past 14 days, he or she may test positive with some RDTs even if he or she no longer has malaria. The test works by detecting an antigen, a substance in the blood produced by malaria parasites that remains in the body for some time even after the parasitaemia has been cleared. PfHRP2 antigen can remain in the blood for 2 weeks or more after all the parasites have been killed.

Q. What if I do not have buffer, can I use plain water or some other liquid to perform an RDT?

Ans. No. Buffer is the only liquid that will work.

Q. What if I do not have an alcohol swab, can I use cotton wool and sterilizing alcohol (spirit) to clean the patient's finger?

Ans. Yes, you can use cotton wool and sterilizing alcohol instead of an alcohol swab. You should not blow on the finger or dry it with anything else.

Q. What if I do not have lancets but have all other materials I need, can I still do the test?

Ans. If you do not have lancets, you can use a sterile hypodermic needle from an unopened package to do the finger-prick. Once you have used the needle, you must discard it in your sharps box just as you would a lancet. The essential thing is that the instrument must always be sterile and unused.

Q. What if the RDT result is negative but the patient still asks me for malaria medication?

Ans. If you *strongly* suspect there is something wrong with the test that gave a negative result, you may repeat the test once using a new RDT, but this should *rarely* be necessary. If the second test is also negative, the patient's symptoms are probably due to some other illness. When a patient has symptoms consistent with *severe* malaria, it is appropriate to treat with antimalarial drugs as per national drug policy *while referring* them to the nearest healthcare facility for further assessment. Delayed treatment of severe malaria may result in death, and RDT results are occasionally wrong.

Q. How long will results remain visible in the test window?

Ans. The results remain visible for at least one hour after testing, but the RDT should be read as early as possible to the time stated in the instructions (e.g., 15 minutes).

Q. Does the test detect all kinds of malaria or just falciparum malaria?

Ans. Even though single kit specific for *Plasmodium falciparum* malaria is available that captures HRP2 antigen, now-a-days combo-kits are operationalized that detects both falciparum (based on HRP2 antigen) and non-falciparum malaria (based on pLDH specific for *P. vivax*).

(Source: https://www.who.int/malaria/areas/diagnosis/rapid-diagnostic-tests/generic_PfPan_training_manual_ web.pdf)

Table 2. Persistence of antigenemia vis-à-vis parasitaemia over subsequent days in *Plasmodium falciparum* malaria cases treated with chloroquine for data based in malaria-endemic block of Assam, North-East India*

Day	Microscopic result parasite density range (µl)	ParaSight-F test kit result on follow up day of
0	6400 – 51,200	+ve
1	2500 – 36,800	+ve
2	800 – 12,800	+ve
3	-ve	+ve
4	-ve	+ve
5	-ve	+ve
6	-ve	+ve
7	-ve	+ve
8	-ve	-ve
9	-ve	-ve

*Data based on 9 follow-up cases on consecutive days. Source Reference [14].

implemented in the Indian national control programme incorporate antibodies to P*f*HRP-2 (specific for *P. falciparum*) and pLDH (specific for non-falciparum malaria). Based on comparative performance, number of RDT products have been approved enabling a policy of parasite-based diagnosis prior to treatment [22]. RDTs, nevertheless are less sensitive to molecular diagnostic methods, heat sensitive, and have reduced sensitivity to non-falciparum malaria.

Molecular diagnostic methods

While clinical diagnosis of malaria is subjective that mimics many other illnesses, both standard microscopic examination as well as RDTs are studded with number of shortcomings including failing to detect low-density parasitaemia (<100 µl) considered vital to the programme in the given malaria elimination initiative (Table 3). Instead, molecular techniques are significantly more sensitive than the traditional diagnostic methods like microscopy and RDTs for plasmodium species identification and can detect low parasitaemia with certainty. The parasite burden due to asymptomatic malaria/sub-patent parasitaemia is huge and remains poorly addressed [23]. Host of modern technologies seem to offer added advantages but remain laboratory-based assays requiring considerable skill and equipment beyond reach in field conditions at point-of-care in resource-poor settings. Molecular techniques which are commonly employed for diagnosis of malaria include conventional Polymerase Chain Reaction (PCR) and allied assays, viz., nested PCR, real-time quantitative PCR (qPCR), and multiplex PCR associated with much greater sensitivity (Table 4).These technologies have gained significance for higher sensitivity as low as 0.02 parasites/µl(sub-microscopic malaria) particularly in identification of mixed infections as well as emerging zoonotic *P. knowlesi* malaria making inroads in many Southeast Asian countries [24,25]. Many more cases have been detected by application of PCR that are normally missed by conventional microscopy [26,27]; qPCR, instead hold advantages over other molecular techniques providing quantification of parasitic densities [28]. Along with these contemporary methods, many other innovative techniques have emerged, viz., reverse transcription-polymerase chain reaction (qRT-PCR) [29], loop-mediated isothermal amplification (LAMP) [30, 31], Aptamer-based electrochemical biosensor (aptasensor)for malaria detection by impedance spectroscopy [32,33], Gazelle device - hemozoin-based malaria diagnostic assay overriding *pfhrp*2/3 gene deletions [34], flow cytometry [35], and microarray [36] as forthcoming assays which hold promise for greater sensitivity to diagnose sub-patent parasitaemia, but specific guidelines are deemed necessary for wider applications that are doable and sustainable in resource-poor settings.

Conclusions

Successful diagnostic tools require high sensitivity, reliability, and short period intervals to process samples and be cost-effective [37]. Early and accurate diagnosis of malaria is of paramount importance to ensure appropriate administration of radical treatment to reduce morbidity minimizing spread of drug-resistance [38]. The development and rollout

Table 3. Relative utility of different techniques for diagnosing *Plasmodium* infection in blood*

Parameter	Microscopy	PCR	Fluorescence	RDT
Sensitivity (parasites/ul)	>100	5	50	>100
Specificity	All *Plasmodium* species	All *Plasmodium* species	*Plasmodium falciparum* (others difficult)	*Plasmodium falciparum* and *P. vivax*
Parasite density	Yes	No	No	No
Time for result	30-60 minutes	24 hours	30-60 minutes	20 minutes
Skill level	High	High	Moderate	Low
Equipment	Microscope	PCR apparatus	QBC apparatus or direct fluorescence microscope	Kit only
Cost/test	Low	High	Moderate/low	Moderate

*Adapted from source Reference [12]

Table 4. Comparative operational performance of allied molecular techniques*

Diagnostic method	Operational features	Performance	Advantages	Disadvantages
Nested PCR	Two sets of primers used in successive reactions, therefore, more expensive, time consuming and potential contamination than single-step PCR.	Limit of detection at least 6 parasites/µl for blood spots.More sensitive than single-step PCR for fourPlasmodium species. Hands-on time to result: 3 h; total time: 10 h	Simple, it reduces the degree of non-specific binding. The specificity of the PCR reaction is enhanced by reducing the non-specific binding with the help of the two sets of primers.	Time consuming,needs more reagents such as extra set of primers, high chance of contamination.
Multiplex PCR	Simultaneous multiplex PCR to detect the presence of multiple Plasmodium species.	Limit of detection: 0.2–5 parasites/µl. Hands-on time to result: 2 h; total time: 4.5 h	More information with less sample, cost effective, time saving, high accuracy, less pipetting errors, less contamination.	Low amplification efficiency, complex, variability in efficiency in different templates and poor universality.
Quantitative PCR	Rapid amplification, simultaneous detection and quantification of target DNA by use of specific fluorophore probes.	Limit of detection: 0.02 parasites/µl for genus-level identification, 1.22 parasites/µl for *P. falciparum* detection. Hands-on time to result: 1 h; total time: 2.5 h	Fast, efficient, and gives a qualitative result.	It is not cost effective and complex due to simultaneous thermal cycling and fluorescence detection.
CLIP-PCR	Highly sensitive method, rRNA of the plasmodium parasite can be released from the blood and then captured onto 96-well plates. Finally, quantified through the number of ligated probes which bound to it.	Capture and ligation probe PCR(CLIP-PCR) which enables to detect the parasite density in blood as low as 0.01 parasites per microliter of blood.	CLIP-PCR is highly sensitive and can detect malaria concentrations as low as 0.01 parasitized cells/µl of blood.	Expensive and complex.
LAMP	Boil-and-spin extraction can be used, with amplification by isothermal method. Result determined by turbidity or fluorescence. Sensitivity increases by including mitochondrial targets. Genus-level targets, *P. falciparum* and *P. vivax*.	Limit of detection: 0.2–2 parasites/µl. Results within 30 min with a tube scanner.	LAMP is cost-effective and requires minimal capital equipment investment.	Restricted availability of reagents and instruments, no multiplex capability and limitations related with primer design.Does not allow the inclusion of an internal PCR inhibition control (IC).

*Source Reference [37]

of RDTs have helped interrupt transmission and proven boon to the control programme in reducing malaria-attributable mortality in high-risk areas/outreach population groups. India is reporting steady decline in cases and fast approaching towards malaria elimination [39]. Time lag between blood-smear collection and availability of result is reduced to nil overriding operational constraints related to difficult terrain, poor public transportation and other communication means and high vacancy/attrition of laboratory technicians/skilled workforce. Despite their added advantages, light microscopy remains the gold standard for diagnostic confirmation, while RDTs have made presumptive treatment a thing of the past helping reduce drug pressure [40]. Nevertheless, while both microscopy and RDTs are still the mainstay of malaria diagnosis in basic healthcare services in the control programme, molecular aided tools are utilized in advanced healthcare systems as part of malaria diagnostic protocol [41]. In the elimination settings, when malaria infections get scarce, molecular methods would be of required to complement field diagnostics (microscopy and RDTs) to identify asymptomatic carriers/sub-microscopic parasitaemia which is critical to the success of the elimination programme. The future of diagnostic assays holds in devising non-invasive methods to reach confirmed diagnosis, viz., detecting specific thioether levels in human breath, bio-markers in saliva and urine examination,however, these are still in infancy. Future work should facilitate the development of improved RDT platforms for *P. falciparum* that surmount the issue of *pfhrp*2/3 gene deletionsas well as low-density infections of non-falciparum malaria parasite species in particular [42], and those which are more robust with much longer shelf-life under adverse environs. Maintaining quality control forbidding circulation of counterfeit supplies and strengthening surveillance are just as vital to success of the control programme aiming malaria elimination [43].

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11

Evolution and spread of drug-resistant malaria: the stumbling block to malaria elimination

Introduction

North-East (NE) India is co-endemic for *Plasmodium falciparum* and *P. vivax* malaria; both occur in abundance, but distribution and parasite proportions varied across its landscape [1]. Yet, *P. falciparum* is by far the predominant infection (90%), the remaining are *P. vivax* cases. NE is sparsely populated vast geographical stretch (constituting ~4% of Indian population) but contributes 10-12% of *P. falciparum* cases and 20% of malaria-attributable deaths reported in the country each year. The region is prone to focal malarial outbreaks which are largely ascribed to rise in *P. falciparum* cases transmitted by *Anopheles minimus* and *An. baimaii*, the most efficient mosquito vector species maintaining perennial transmission [2]. Resurgence and extended morbidity/attributable mortality were in part attributed to *P. falciparum* drug-resistant malaria first recorded in Karbi Anglong, Assam way back in 1973 that soon became wide spread [3,4]. *P. falciparum* cases were seen increasing each passing year most of which were concentrated in tribal dominated states/districts of NE, eastern and central India. To contain its spread, *Plasmodium falciparum* Containment Programme (PfCP), a jointly funded initiative of the World Health Organization and Swedish International Development Agency (WHO/SIDA) was launched in high-risk states/districts of the country [5]. After 10 years of operation (1978-1988), solely based on intensive disease surveillance and focussed spraying, drug-resistant foci were diluted to great extent. Yet march of *P. falciparum* malaria continued relentlessly due to myriad of reasons inter alia decreased sensitivity to commonly used antimalarial drugs [6]. The problem was compounded particularly along international borders to NE that it shares with Myanmar and Bangladesh wherein health infrastructure was far from adequate to address the issue in its entirety for lack of coordinated action across borders [7]. Prior to 1970s, the studies on therapeutic efficacies of antimalarials were few and far resulting in inadequate treatment for long resulting in pool of drug-resistant parasites unchecked. Included in this communication is an account of monitoring therapeutic efficacies of antimalarials in space and time that helped evolve drug-policy to contain spread of drug-resistant malaria.

Antimalarial drugs: armamentarium and therapeutic efficacies

The cessation of World War II helped control malaria by two major outcomes, i.e., DDT for vector containment and chloroquine (CQ) for treatment of malaria. Ever since inception of the control programme in 1953, vector control and treatment of cases remained the key interventions against malaria.Large scale application of both these intervention tools helped control malaria near elimination in 1960s. CQ was the choice drug for clearance of the asexual stages of both *P. falciparum* and *P. vivax* and continued to be used for long inclusive of presumptive treatment of clinical cases [8]. In the present-day context, while

CQ remains to be susceptible for treatment of *P. vivax* (barring a few sporadic reports of resistance) malaria [9], it has become an obsolete drug for treatment of *P. falciparum* malaria across India [10,11]. Monitoring of therapeutic efficacies in some high-risk districts of Assam revealed that CQ resistance (despite enhanced dosage of 25 mg/kg of body weight) was widely prevalent and clinical response was much less than adequate mandating change in drug-policy (Table 1). Among available therapeutic options, sulfadoxine-pyrimethamine (SP) was chosen as alternate therapy to CQ in 2004 but enforced in select districts reporting CQ-resistant malaria. The curative efficacy of SP, however, was only short-lived resulting in two-fold rise in cases within two years' time interval [12].There were signs of emerging SP resistance evidenced by late treatment failure, but it needs to be revalidated by additional data sets.

Table 1. The clinical and parasitological response to sequential therapy of *Plasmodium falciparum* malaria with chloroquine, sulfadoxine-pyrimethamine and quinine for data based in malaria-endemic blocks of Assam, North-East India. [See Box for given criteria assessing response to therapy]

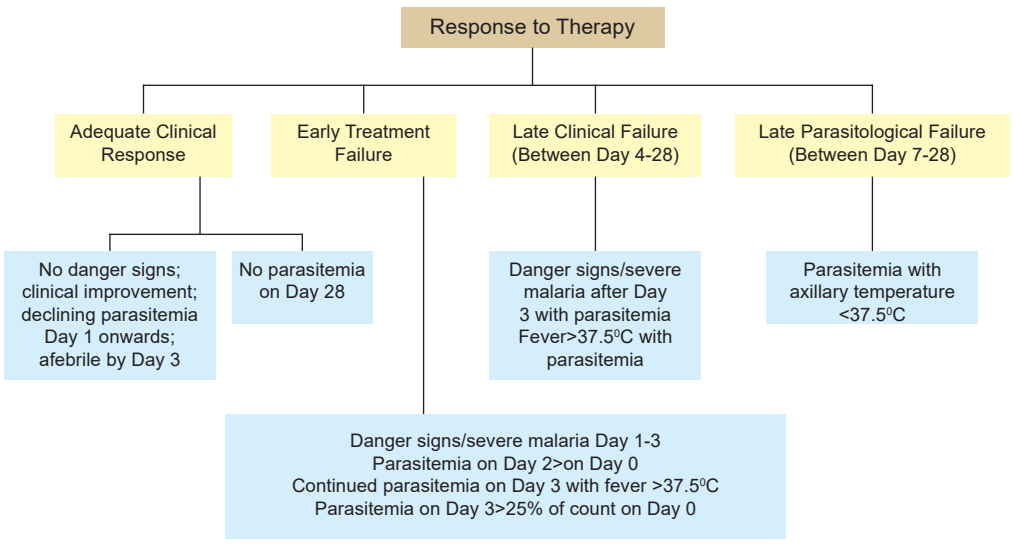
Treatment response*	No. and (%) of cases in different sites (study period)			All sites combined
	Sonapur (July-Aug., 2001)	Boko (July-Sept., 2002)	Tamulpur (July-Aug., 2000)	
Chloroquine (CQ)				
Early treatment failure (ETF)	5 (6.9)	1 (1.7)	0 (0)	6 (4.2)
Late treatment failure (LTF)	19 (26.0)	5 (8.7)	5 (38.5)	29 (20.1)
Adequate Clinical Response (ACR)	49 (67.1)	52 (89.7)	8 (61.5)	109 (75.7)
Total	**73 (100)**	**58 (100)**	**13 (100)**	**144 (100)**
Sulfadoxine-Pyrimethamine (SP)				
Early treatment failure (ETF)	0 (0)	0 (0)	1 (25)	1 (2.9)
Late treatment failure (LTF)	2 (8.3)	0 (0)	0 (0)	2 (5.9)
Adequate Clinical Response (ACR)	22 (91.7)	6 (100)	3 (75)	31(91.2)
Total	**24 (100)**	**6 (100)**	**4 (100)**	**34 (100)**
Quinine				
Early treatment failure (ETF)	0 (0)	0 (0)	0 (0)	0 (0)
Late treatment failure (LTF)	1 (50)	0 (0)	0 (0)	1 (33.3)
Adequate Clinical Response (ACR)	1 (50)	0 (0)	1 (100)	2 (66.7)
Total	**2 (100)**	**0 (0)**	**1 (100)**	**3 (100)**

*Adequate Clinical Response (ACR), Late Treatment Failure (LTF), Early Treatment Failure (ETF) as per WHO guidelines (1996). Source Reference [10]

Instead, the advent of artemisinin-based derivatives, viz., artemether, α–β arteether, artesunate, dihydroartemisinin, was laudable development raising new hopes for treatment of drug-resistant malaria. Among these, indigenously developed α–β arteether (administered intramuscularly @ 150 mg once daily for three consecutive days) formulation was evaluated to be highly efficacious fast acting schizonticidal and was assessed to be convenient to administer for treatment of severe and complicated cases [13-15]. Extended follow up investigations based in Assam revealed that in most cases (83%, 34/41), asexual parasitaemia was cleared by day-1 (within 24h), rest turned aparasitaemic within 48h of administration [13]. The use of artemisinin monotherapies, however, was abandoned

soon in 2009 anticipating resistance sooner than later [16]. Consequently, artemisinin combination therapies (ACTs) were advocated by combining artemisinin derivatives (short-acting) with long-acting partner drug to clear the residual parasites. Six different combination therapies were approved by the World Health Organization: these included artemether - lumefantrine (AL), artesunate-amodiaquine (AS-AQ), artesunate - mefloquine (AS-MQ), artesunate - pyronaridine (AS-PY), artesunate + sulfadoxine - pyrimethamine (ASP) and dihydroartemisinin - piperaquine (DHA-PPQ) [17]. Among these available options, artesunate + SP (ASP) was adopted, SP being already in the programme,initially restricting its implementation in districts reporting CQ resistant malaria and later rolled out for treatment of every single case of *P. falciparum* throughout the country. Therapeutic assessment of this combination was held efficacious (treatment success >95%) and safe in various epidemiological settings across India resulting in notable transmission reduction each passing year [18,19]. In due course of time, resistance to this combination had also surfaced in 2013 first detected in border districts to Bangladesh in NE region [20], which led to change in drug policy replacing ASP with artemether + lumefantrine (AL), the latter was assessed to be far more superior for its clinical efficacy (Table 2) [21]. At present AL is in force specific to NE India for which monitoring therapeutic efficacy is continuing before its clinical efficacy fall short of cut off percentage of 98% cure rate.

Box: Criteria for assessing therapeutic efficacies of antimalarial drugs for treatment of malaria



Source: https://www.malariasite.com/

North-East region: the corridor for spread of drug-resistant malaria to peninsular India and beyond

Most districts of NE particularly those sharing border with Myanmar and Bangladesh are hyperendemic for malaria contributing bulk of *P. falciparum* cases (>90%) [22].These are also the districts reporting focal outbreaks associated with marked increase in cases

Table 2. Therapeutic efficacy of antimalarials for treatment of *Plasmodium falciparum* malaria in North-East India

Anti-malarial treatment	District (State)	Study period	No. and (%) of subjects parasitaemic on follow up day of						
			Day 0	Day 2	Day 3	Day 7	Day 14	Day 21	Day 28
CQ[1]	Kamrup & Nalbari (Assam)	2000 - 2002	144 (100)	23 (15.9)	17 (11.8)	5 (3.5)	11 (7.6)	9 (6.3)	3 (2.1)
CQ/SP[2]	Kamrup & Nalbari (Assam)	2000 - 2002	34 (100)	4 (11.8)	2 (5.9)	1 (2.9)	1 (2.9)	0	0
SP[3]	Kamrup (Assam)	2004	54 (100)	28 (50.9)	4 (7.3)	0	1 (1.8)	4 (7.3)	4 (7.3)
$\alpha - \beta$ arteether	Kamrup & Darrang (Assam)	1995 - 1996	41 (100)	6 (14.6)	0	0	0	0	0
AS + SP[4]	Darrang (Assam)	2005	51	No data	0	0	0	0	3 (5.9)
	Nalbari (Assam)	2006	53	No data	1 (1.9)	0	0	4 (7.5)	2 (3.8)
	West Garo Hills (Meghalaya)	2007	54 (100)	No data	0	0	1 (1.8)	2 (3.7)	0
AL[5]	Kamrup (Assam)	2007	53 (100)	10 (18.8)	0	0	0	0	0

[1]CQ = chloroquine, [2]CQ/SP = sequential therapy with sulfadoxine-pyrimethamine for chloroquine resistant cases, [3]SP = sulfadoxine - pyrimethamine, [4]AS+SP = artesunate + sulfadoxine-pyrimethamine, [5]AL = artemether + lumefantrine. Source Reference [12]

and attributable deaths; each death was confirmed solely due to *P. falciparum* infection [23,24]. Borders are porous and cross-border migration is unstoppable permitting mixing of parasite strains resulting in fulminating outbreaks on account of varied immune status and lack of access to healthcare services. Infrastructure is too far weak; most villages are difficult to reach due to lack of motorable roads leaving many cases unattended for poor disease surveillance. Disease transmission is persistent, and interventions are far from adequate due to lack of coordinated action across borders resulting in pool of parasite reservoir left unattended. The indigenous population groups living in these pockets are largely illiterate little aware of disease prevention and cure often do not seek treatment in time least the compliance. It is postulated that drug-resistant malaria first reported in NE India is the outcome of movement of strains originating from Thai/Cambodia border on the way to Burma (presently Myanmar) and onward journey to NE India sharing vast international border (Figure 1). The drug-resistant strains apparently have propagated in the NE region having shared ecology and efficient disease vector species on both sides of the border held high-risk for malaria. Disease transmission was intense and drug-resistant foci had apparently multiplied manifold [25-27]. NE borders are heavily fortified with defence posts having personnel strength hailing from various states of India with varying endemicity. The security personnel are equally exposed to malarial infection and bear the brunt often associated with high morbidity and fatal outcome. High degree of malaria morbidity has been documented in Central Reserve Police Force (CRPF) Battalions outposted along international border posts of NE (Table 3) [28]. Similar levels of morbidity have been reported in allied defence services specific to NE region [29,30].

Table 3. Malaria-attributable morbidity and mortality in Central Reserve Police Force Battalions (CRPF BNs) located at border posts in the North-Eastern states of India (1999-2001)*

State	1999				2000				2001			
	No. of BNs (pop.)	No. of fever cases	No. of malaria cases	No. of deaths	No. of BNs (pop.)	No. of fever cases	No. of malaria cases	No. of deaths	No. of BNs (pop.)	No. of fever cases	No. of malaria cases	No. of deaths
Assam	20 (18740)	415	152	5	19 (17803)	379	117	4	19 (17803)	517	187	10
Tripura	9 (8433)	327	48	4	10 (9370)	536	104	6	10 (9370)	554	152	1
Manipur	4 (3748)	19	4	3	4 (3748)	16	16	3	4 (3748)	29	13	1
Meghalaya	1 (937)	0	0	0	1 (937)	67	67	1	2 (1874)	168	63	0
Nagaland	5 (4833)	47	41	0	4 (3748)	20	14	0	4 (3748)	10	5	0
Mizoram	1 (937)	37	37	1	1 (937)	29	3	0	1 (937)	48	17	0
Arunachal Pradesh	1 (937)	12	6	0	2 (1874)	35	20	0	2 (1874)	17	6	2

*Source Reference [28]; BN = Battalions

These security personnel are routinely outposted across states many of which are turned carriers of drug-resistant strains facilitating spread to peninsular India. Drug-resistant malaria is presently widespread; relative risk, however, varied across the landscape with high to moderate risk in tribal population concentrations in the NE, east and central India (Figure 2).

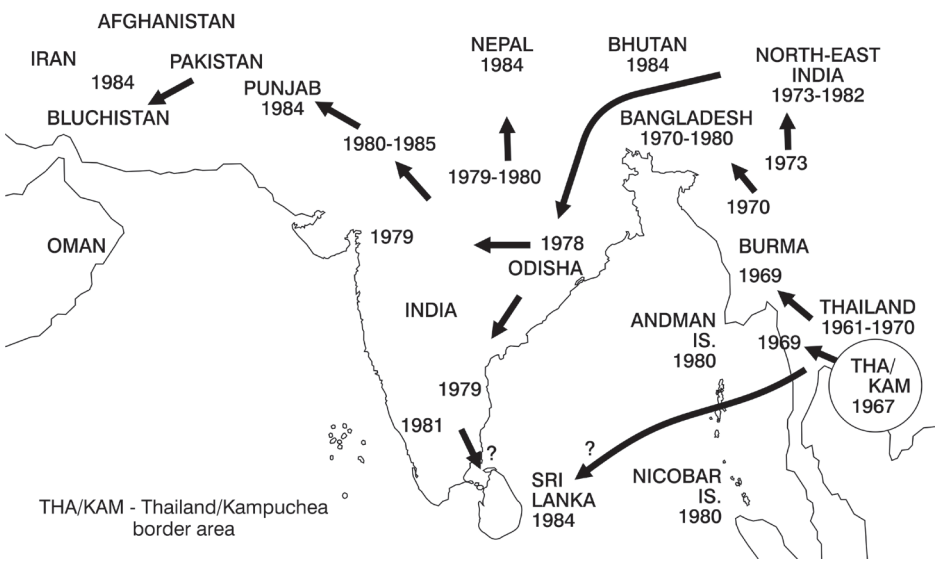


Figure 1. Origin and route of spread of drug-resistant malaria in India and westwards. (Courtesy: V.P. Sharma)

Figure 2. Distribution and relative risk of drug-resistant malaria in India. (Courtesy: V.P. Sharma)

Treatment policy and changing transmission dynamics

In the Indian national malaria control programme, detection and treatment of cases is based on active disease surveillance (fortnightly domiciliary visits) and passive case detection (self-reporting at the nearby malaria clinic). Active case detection can best be described as fragmented for missing many more cases besides there being a wide gap (two weeks or more) between presumptive (later discontinued) and radical treatment resulting in extended morbidity. Many high-risk villages are remote (>5 km from nearest healthcare facility) and rendered inaccessible on account of incessant rains and flood, lack of motorable road and security concerns leaving them untreated months together [31]. During the high transmission seasons (April-September), case load in practice remained too heavy on the

assigned laboratory technician adding to the woes for providing test result in due time often amounting to misdiagnosis. Antimalarial drug-policy came into being much too late; the first such policy came in force in 1982 which relied heavily on CQ as the choice drug for treatment of *P. falciparum* cases and *P. vivax* cases alike [32]. Later in 1995 the existing drug policy was upgraded permitting SP (1500 mg sulfadoxine +75 mg pyrimethamine adult stat dose) in CQ-resistant pockets, following which review of the drug-policy was mandated every two years in keeping with the changing scenario. Taking stock of fast emerging multi-drug resistant strains, artemisinin-based combination therapies (ACTs) came in limelight in 2004 replacing previous regimens.The advent of artemisinin (so called wonder drug) revolutionized the treatment options for its rapid action and tolerability. It is derived from herb popularly known as sweet-worm wood commonly found in hill tracts of NE states (Figure 3). It was in 2007 that presumptive treatment based on clinical diagnosis was discontinued, instead in so far as possible confirmed diagnosis by microscopy and/or rapid diagnostic kits (RDKs) was mandated for radical cure. In 2010, ACT (ASP), initially restricted to few districts, was rolled out (except in pregnancy in which case it was approved for use in 2nd and 3rd trimester) throughout the country for treatment of every confirmed case of *P. falciparum*. In 2013, declining efficacy to this combination also surfaced along NE borders and was replaced with AL (artemether lumefantrine) specific to NE region [19,20], and continues to be in force (Figure 4).

Drug failure: genesis and implications

In contrast to *P. vivax* (BT - benign tertian malaria), *P. falciparum* (MT - malignant tertian malaria) is fast evolving parasitic infection invading all ages of RBCs affecting multiple organs often with fatal outcome, if not treated in time [33]. Clinical presentation may vary

Figure 3. *Artemisia annua*, also known as sweet-wormwood is native to temperate Asia. An extract of *Artemisia annua* called artemisinin is a medication used to treat malaria that helped saved millions of lives. Discovery of artemisinin and its antimalarial properties by the Chinese scientist, Tu Youyou, led to award of the Nobel Prize in Physiology and Medicine in 2015.

widely ranging from no fever to high fever (depending upon endemicity) associated with disorders including altered sensorium (cerebral involvement), acute anaemia, liver and kidney failure.

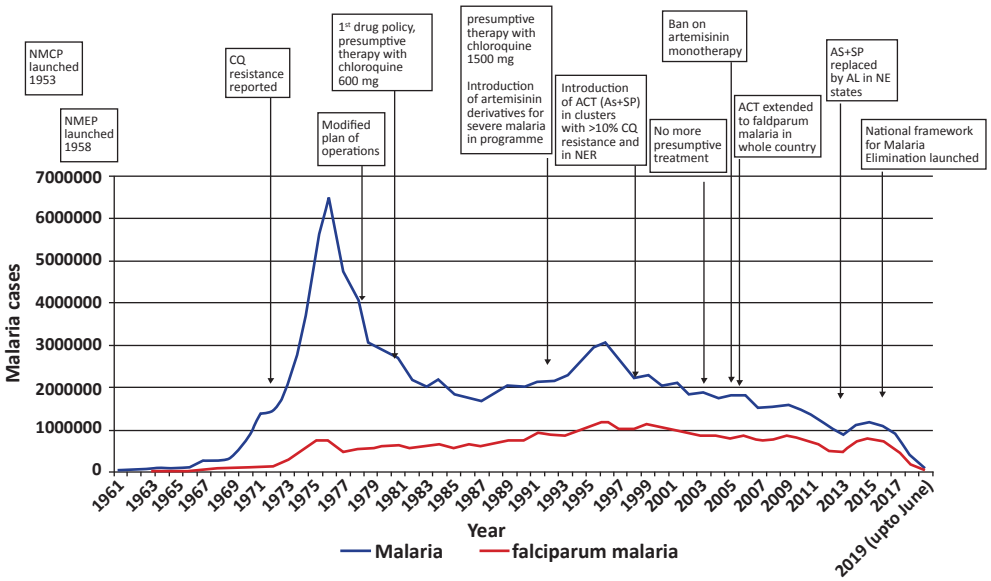


Figure 4. Malaria transmission and evolution of antimalarial drug policy in India (1961-2019). Acronyms NMCP, NMEP, CQ, ACT, AS+SP, AL and NER denote National Malaria Control Programme, National Malaria Eradication Programme, chloroquine, artemisinin-based combination therapy, artesunate+sulfadoxine-pyrimethamine, artemether+lumefantrine and North-East Region respectively (Courtesy: AnupkumarAnvikar, National Institute of Malaria Research, New Delhi).

Early case detection and prompt treatment (EDPT) is held vital to reduce morbidity which itself is a challenging task given the enormity of disease burden and varied landscape. It is hypothesized that reduced therapeutic efficacy is the outcome of development of hidden parasite reservoir on account of multiple reasons: (i) broken disease surveillance reporting fraction of true incidence; WHO estimates are manifold [34], (ii) delayed diagnosis and treatment on account of late reporting, (iii) irrational use of antimalarials for long inclusive of presumptive treatment years together, (iv) non-compliance of treatment regimens particularly in high-risk are as permitting development of gametocytaemia, (v) huge private sector giving symptomatic treatment not necessarily in conformity with drug-policy, (vi) inadequate vector control intervention and poor access to healthcare services in the periphery; all these constraints have compounded resulting in persistent transmission and proliferation of drug-resistant strains. Consequently, there has been rise in proportions of *P. falciparum* cases each passing year from erstwhile 29% in 1985 to 63% in 2020 (Figure 5). Malaria outbreak investigations revealed that lack of interventions years together had resulted in build-up of vector density well beyond threshold coupled withthe availability of gametocyte pool amounting to heavy transmission [35,36]. Focal disease had been recurring amidst chaos and panic more so in forest-fringe/cross-border population groups associated with significant rise in *P. falciparum* cases [25]. Morbidity and attributable mortality have been alarming well above the carrying capacity of healthcare

facilities (Figure 6). Populations of *P. falciparum* are genetically diverse having implication in developing candidate vaccines and exhibited varied levels of host-parasite response in relation to drug-sensitivity status [37-39]. For instance, it was observed that 'tea-tribes' (conserved community) in Assam were highly susceptible to standard regimen of CQ [40]; most cases (even with parasite density ranging from 1,00,000 - 4,00,000 per cubic ml) were turned aparasitaemic by day- 4 of follow up (Table 4). Malaria-attributable deaths, however, were attributed to late reporting and consequent delayed diagnosis [41]. Instead, indigenous tribal population groups having distinct genetic identity exhibited high levels of resistance to arsenal of antimalarials [10]. Drug-resistance has been invariably associated with genetic mutations in parasite populations helping evade action of antimalarials. For instance, whereas CQ resistance has been linked to K76 mutation in the *P. falciparum* chloroquine resistance transporter (*pfcrt*) gene; temporal increase in frequencies in *pfcrt* as well as *pfmdr*12-loci have been associated with higher levels of resistance [42,43].

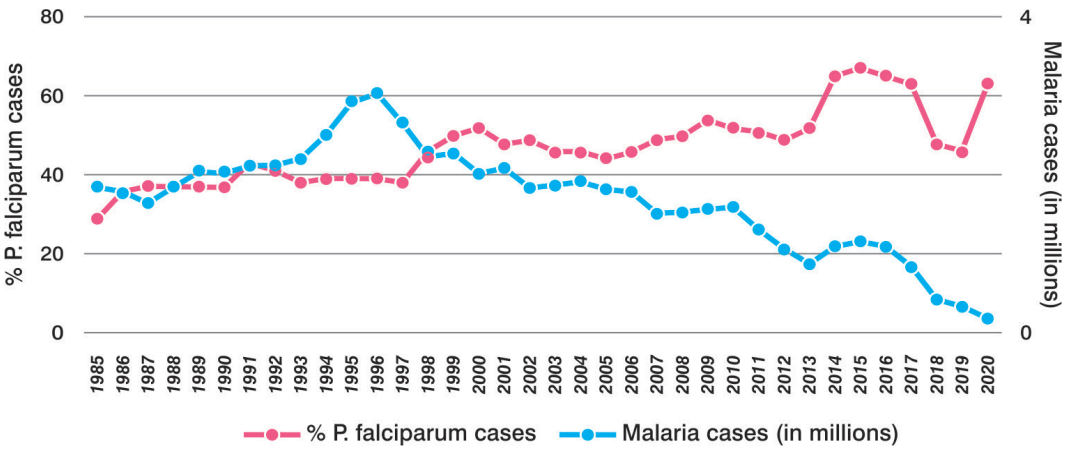


Figure 5. Rising proportions of *Plasmodium falciparum* cases in India. Data source: National Vector Borne Disease Control Programme. While malaria cases have consistently declined from 1996 onwards, *P. falciparum* prevalence have steadily increased from 29% in 1985 to 64% in 2020.

Figure 6. Hospital full to the capacity with clinical malaria patients reporting fever with severe complications in outbreak-affected block ofAssam, North-East India.

Table 4. Results of a simplified *in vivo* 3-day test for chloroquine sensitivity in *Plasmodium falciparum* for data based in Tarajulie Tea Estate (Tezpur Circle), Assam, North-East India (May-September 1992)

S. No.	Age in years/sex	Asexual parasite count/mm³ of blood at different days interval			Degree of resistance*
		Day 0	Day 4	Day 7	
1	43/F	15,600	13,280	14,800	RIII
2	5/M	8.960	Neg	Neg	S/RI
3	50/F	35,200	Neg	Neg	S/RI
4	30/M	3,360	Neg	Neg	S/RI
5	12/F	848	Neg	Pfg	S/RI
6	24/M	14,080	Neg	Pfg	S/RI
7	56/M	15,600	Neg	Neg	S/RI
8	25/F	23,200	Neg	Neg	S/RI
9	10/F	6,400	Neg	Neg	S/RI
10	2/F	14,200	Pfg	Pfg	S/RI
11	35/M	5,600	Neg	Neg	S/RI
12	50/F	4,400	Pfg	Pfg	S/RI
13	15/M	50,000	Neg	Pfg	S/RI
14	10/M	6,800	Pfg	Neg	S/RI
15	15/F	1,824	Neg	Neg	S/RI
16	4/M	20,000	Pfg	Pfg	S/RI
17	3/M	5,640	Neg	21,600	RI
18	13/M	1,13,600	Neg	Pfg	S/RI
19	45/M	24,400	Neg	Neg	S/RI
20	70/F	8,800	Pfg	Pfg	S/RI
21	50/M	5,600	Neg	Neg	S/RI
22	15/F	60,000	Neg	Neg	S/RI
23	24/F	10,240	Neg	6,400	RI
24	22/M	31,600	Neg	Neg	S/RI
25	34/M	1,45,600	Pfg	Pfg	S/RI
26	12/M	10,560	Neg	Neg	S/RI
27	10/F	6,640	Neg	Pfg	S/RI
28	4/M	15,200	Neg	Pfg	S/RI
29	3/F	4,80,000	Neg	Pfg	S/RI
30	5/F	3,20,000	Neg	Neg	S/RI
31	30/M	4,800	Neg	Neg	S/RI
32	3/F	11,840	5,760	8,640	RIII
33	30/M	80,000	11,200	28,800	RII
34	52/F	10,400	Neg	5.600	RI
35	4/M	55,200	Pfg	Neg	S/RI
36	7/M	4,000	Neg	1,120	RI
37	30/M	10,240	55,600	Referred	RIII
38	4/M	42,400	Neg	Pfg	S/RI
39	75/M	35,600	Pfg	Neg	S/RI
40	2/F	1,20,800	Neg	Neg	S/RI

*Subjects with parasite clearance within 7 days and those with recrudescence on day 7 were categorized as S/RI and RI respectively. Follow up subjects with marked reduction of asexual parasitaemia but no clearance was taken as RII, and those with no marked reduction were classified as RIII. The simplified follow up protocol for monitoring drug sensitivity studies was later discontinued in 2003 and replaced with extended therapeutic efficacy follow up investigations categorized as Adequate Clinical Response (ACR), Late Treatment Failure (LTF) and Early Treatment Failure (ETF). S = sensitive status while RI, RII and RIII denote increasing order of drug-resistance. Neg = blood-smear negative for malaria parasite, Pfg = blood-smear with *Plasmodium falciparum* gametocytes. Source Reference [40]

Similarly, resistance to SP has been associated with progressive increase in point mutations in genes for dihydrofolate reductase (DHFR) and dihydropteroate synthetase (DHPS) enzymes of the parasite having epidemiological significance [44]. By each successive generation, while susceptible parasite populations were eliminated, resistant populations proliferated and accumulated over space and time [45]. There are indications of developing quinine resistance evidenced by whole blood concentrations as well as associated genetic markers [46]. Molecular surveillance of these markers can help predict emerging drug-resistance and formulate drug-policy in time and place before they become widespread.

Future challenges and the way forward

Among several landmark discoveries for malaria control in the twentieth century, the development of artemisinin derived from the Chinese herb Qinghaosu- also known as sweet-wormwood (*Artemisia annua*) has revolutionized malaria treatment helping achieve malaria elimination in many endemic countries [34]. Several ACTs are made available and have been endorsed by WHO giving options for treatment by alternate regimens failing resistance to the partner drug [47]. The relentless march of *P. falciparum* is, however, continuing associated with increasing levels of drug-resistance for which periodic monitoring of therapeutic efficacies should be the guiding principle. Artemisinin resistance is emerging threat globally calling for continuing efforts inventing newer molecules which are more potent and affordable [48]. There are already signs of decreasing sensitivity to artemisinin in east and NE India characterized by delayed therapeutic response [49,50]. Equally important would be to strengthen healthcare system ensuing early diagnosis and treatment in the periphery hard-to-reach population groups to mitigate the transmission risk. What even more important is the treatment compliance something that is difficult to achieve in population groups which are most at risk. Availability of fixed-dose combination (FDC) therapies hold promise [51], yet timely compliance is anybody's guess. The rollout of ACTs has certainly helped achieve substantial transmission reduction yet much more can be achieved by ensuring sustained logistics supplies prioritizing high-risk areas [52]. There is need to maintain vigil (by means of periodic pharmacovigilance) preventing circulation of counterfeit drugs which can jeopardize the gains thus made in defeating malaria amounting to huge economic losses [53]. Training and re-orientation of health personnel inclusive of public and private sector alike hold key for reaching confirmed diagnosis aided by modern tools, adherence to treatment schedules and ensuring much needed community compliance. It is high time to stay step ahead of emerging resistance for which molecular surveillance along with optimal use of current available and forthcoming drugs are vital to walk the last mile in ending malaria transmission [54,55].

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Vector Control

12

Vector control: a cornerstone intervention for malaria transmission containment, current practices, and emerging technologies

Introduction

In the Indian national malaria control programme, vector control is an integral component to contain transmission for which it relies heavily on insecticide residual spraying (IRS) incurring huge costs [1]. There are multiple vector species transmitting malaria in different geo-epidemiological regions of the country, susceptibility status of which varies to available insecticides [2,3]. Disease distribution and transmission intensities are heterogenous across states with large concentration of cases in the east, east central and north-eastern (NE) states adding up to nearly 80% of disease burden [4]. Malaria is a major public health concern in NE India for contributing substantial number of cases annually, majority of which are due to *Plasmodium falciparum* [5,6]. Ever since inception of the control programme in 1953, DDT continues to be applied specific to NE region for proven efficacy against local disease vector species, *Anopheles minimus* and *An. baimaii* (sibling-species of the *An. dirus* complex), even though DDT is banned for use in public health programmes under Stockholm Convention on Persistent Organic Pollutants (POP). However, decades of applied interventions did not disrupt transmission in large tracts of land with record of recurring disease outbreaks inflicting insurmountable morbidity. Operational costs were seen rising and returns diminishing making it a difficult proposition on account of operational constraints including high refusal rates in communities at stake [7]. Consequently, newer interventions were field evaluated as alternative control options that are community-based, eco-friendly, socially acceptable and sustainable. Given in this chapter is an account of travelogue of applied interventions, highlighting advantages/shortfalls, and outcome aiming to achieve the common goal of ending transmission specific to NE India.

DDT: the choice insecticide

Dichloro-diphenyl-trichloroethane (DDT), had been extensively used in the control programme as residual insecticide and continues to be applied specific to NE India. On account of large-scale application of DDT for years together during 1953-1960s (under vertical control programme), malaria had vanished in large tracts of land and *An. minimus* (the major vector in the valleys) was believed to have disappeared, instead *An. philippinensis* was implicated in ongoing residual transmission [8,9]. The return of malaria post 1970s (decentralized horizontal programme under modified plan of operation) was attributed to developing insecticide resistance which in fact did not exist; instead, *An. minimus* had turned exophilic (resting outdoors) in relation to intensive indoor residual spraying (IRS) operations. Both *An. minimus* (primarily endophilic) and *An. baimaii* (exophilic, forest dweller) remained susceptible to DDT by virtue of their innate abilities of behavioral resistance evading contact with sprayed surfaces till residual effect faded away [10]. As

a matter of policy, normally two consecutive rounds of DDT (50% wp @ 1 gm per square meter) are scheduled corresponding to high transmission season (April – September) few weeks apart, but in practice it was observed that besides missing one round, coverage fell short of target population years together resulting in continued transmission (Table 1) [11]. Situational analysis revealed that spray operations were often late/not done in scheduled time scale and poorly executed (not uniformly applied) for lack of intermediate supervision [12]. Many houses were left unsprayed for not permitting spraying indoors while others found locked due to lack of prior intimation. Co-incidentally, malaria season also overlapped with the festive season having many households (166/869, 19%) mud-plastered both interiors/exteriors as that is routine practice for upkeeping their premises thus rendering spray ineffective amounting to waste of exercise (Figure 1).

Table 1. Malaria transmission intensities and vector control interventions for data based in Assam, North-East India (1998 – 2008)*

Year	Population in millions (% pop affected)**	No. and (%) of blood-smears			% *Plasmodium falciparum* cases of total smear positive cases	Annual parasite incidence (no. of confirmed cases/ 1000 population)	DDT data				No. of death cases
		Examined (% of population checked)	Positive for malaria parasite (%)	Positive for *Plasmodium falciparum* (%)			Targeted population (% coverage)		Quantity used in Kg		
							1st spray	2nd spray	1st spray	2nd spray	
1998	26.12	2441084 (9.34)	94645 (3.87)	54769 (2.24)	57.86	3.61	14658872 (79.67)	Not done	877904	0	34
1999	26.63	2872859 (10.78)	131048 (4.56)	83064 (2.89)	63.38	4.91	15436286 (81.86)	6031876 (76.90)	1081477	363830	111
2000	26.90	2215375 (8.23)	84915 (3.83)	52116 (2.35)	61.37	3.15	14592590 (79.30)	6967822 (61.89)	937238	339453	43
2001	27.32 (39)	2432620 (8.90)	95142 (3.91)	58951 (2.48)	61.97	3.48	15645797 (77.02)	Not done	917766	0	122
2002	27.73 (23)	2325105 (8.32)	89601 (3.85)	55825 (2.40)	62.30	3.21	14008712 (70.80)	5838094 (70.63)	823537	292956	72
2003	27.85 (17)	2133820 (7.66)	76570 (3.85)	48647 (2.27)	63.53	2.74	14144089 (77.45)	7071625 (90.00)	802885	378449	53
2004	28.73 (13)	1853560 (6.45)	58134 (3.13)	41400 (2.23)	71.21	2.02	11842156 (81.00)	Not done	748801	0	52
2005	29.00 (31)	2050261 (7.06)	67885 (3.31)	45453 (2.21)	66.95	3.34	12964867 (78.00)	Not done	797253	0	113
2006	29.32 (33)	2743092 (9.35)	126178 (4.59)	82546 (3.00)	65.42	4.30	12534987 (74.00)	12820065 (91.42)	756540	1381905	304
2007	29.65 (30.71)	2399836 (8.09)	94853 (3.12)	65542 (2.73)	69.09	3.19	13365385 (81.72)	109297784 (75.21)	840431	575991	152
2008	30.59	2687756 (8.62)	83939 (3.12)	58124 (2.20)	69.30	2.74	8427442 (79.00)	7696344 (78.00)	495373	428912	86

*Source, State Health Directorate of Assam; **% population reporting high rise in cases and attributable deaths. Source Reference [11]

Figure 1. Typical household in malaria-endemic village of Assam made of split-bamboo mud-plastered both inside out during local festivals as routine maintenance exercise coinciding with the malaria season.

Paradoxically, while the transmission in large tracts of NE India is perennial [13], three rounds instead of two rounds (each round of six weeks duration) should have been mandated for complete protection uninterrupted against *An. minimus* and *An. baimaii*; both are highly anthropophilic actively foraging human host for bloodmeal and held responsible for focal disease outbreaks evidenced by records of prevalence and incrimination [10,14].

Monitoring insecticide resistance

Insecticide resistance is growing menace globally threatening malaria elimination efforts [15]. Monitoring resistance is critical component of the control programme to keep pace with the changing disease epidemiology and susceptibility status of vector populations. While most disease vectors have become DDT resistant [16,17]; *An. minimus* mosquitoes are assessed to be susceptible to target dose of DDT in space and time, hence recommended to be applied specific to NE India. It was observed that populations of *An. minimus* were fully susceptible to designated concentration of not only DDT (4%) but also to malathion (5%), and pyrethroids including permethrin (0.75%), alpha-cypermethrin (0.10%) and deltamethrin (0.05%) across NE states (Table 2) [10].

Table 2: Insecticide susceptibility status of adult mosquito vector populations of *Anopheles minimus* to diagnostic concentration of insecticides in North-East India

Study location, district, State	Insecticide (diagnostic-conc.)	Study period	No. of mosquitoes exposed (no. of replicates)	No. of mosquitoes knockdown post 60 min exposure	No. of mosquitoes dead post 24 hours exposure	Mortality (%)	Status*
Sonapur, Kamrup, Assam	DDT (4%)	October, 1995	43 (5)	43	43	100	S
Agia, Goalpara, Assam	-do-	October, 1995	13 (1)	13	13	100	S
Sonapur, Kamrup, Assam	-do-	July, 1999	80 (4)	80	80	100	S
Sonapur, Kamrup, Assam	-do-	October, 2001	80 (4)	80	80	100	S
Sonapur, Kamrup, Assam	-do-	Nov., 2005	80 (4)	80	80	100	S
Dalu, West Garo Hills, Meghalaya	-do-	June, 2007	30 (2)	30	30	100	S
Bokajan, Karbi Anglong, Assam	-do-	August, 2008	80 (4)	80	80	100	S
Boginadai, Lakhimpur, Assam	-do-	August, 2009	20 (2)	20	20	100	S
Agia, Goalpara, Assam	-do-	October, 2009	44 (4)	44	44	100	S
Amarpur, South Tripura, Tripura	-do-	October, 2010	10 (1)	10	10	100	S
Sidli, Chirang, Assam	-do-	October, 2012	24 (2)	24	24	100	S
Silachari, South Tripura, Tripura	-do-	Sept., 2012	40 (4)	40	40	100	S
Sonapur, Kamrup, Assam	Malathion (5%)	July, 1999	60 (3)	60	60	100	S
Sonapur, Kamrup, Assam	Malathion (5%)	Nov., 2005	60 (3)	60	60	100	S
Sonapur, Kamrup, Assam	Permethrin (0.75%)	Nov., 2005	40 (2)	40	40	100	S
Sonapur, Kamrup, Assam	Alpha-cypermethrin (0.10%)	August, 2006	80 (4)	80	80	100	S
Sonapur, Kamrup, Assam	Deltamethrin (0.05%)	Nov., 2005	40 (2)	40	40	100	S

*S denotes susceptible status of mosquito population to given insecticide (mortality in control replicates was <5%). Source Reference [10]

Same holds true for *An. baimaii* populations of which are susceptible to all available residual insecticides including DDT [18]. Paradoxically, despite proven efficacy of DDT, IRS operations lacked community support and refused spraying indoors for variety of reasons: (i) do not believe that DDT is effective against biting mosquitoes, (ii) foul smell and spoiling interiors of the house, (iii) fear of adverse events/danger to children, (iv) inconvenient exercise involving removing household goods, (v) rearing silkworms for handlooms, and the like. It was amply clear that IRS operations had become operationally difficult exercise on account of high refusal rates (>50%) by the communities at risk, poor coverages and accessibility to remote/forest fringe areas during high transmission season on account of incessant rains/flash floods, lack of connectivity, and social/ethnic conflicts. Given these facts, it was imperative that alternative interventions are deemed necessary that are doable, community-based, cost-effective, and sustainable for effective vector management.

Insecticide-treated nets (ITNs): an alternative strategy for malaria vector control & impact assessment

Long ago, it was opined that impregnating mosquito nets with insecticide would be more acceptable to the Indian communities than IRS given the cultural practices using net for personal protection against biting insects [19]. As such net ownership in NE India is high but untreated nets were proven to confer only partial protection against malaria [20]. It was prudent that net impregnation of the community-owned nets with suitable insecticide would enhance protection manifold than untreated nets [21]. Large-scale implementation of pyrethroid-impregnated mosquito nets in China and other malarious countries had resulted in optimal outcome for control of malaria [22]. Prompted by the study results, field-evaluation of deltamethrin impregnated nets was conducted in typical malaria-endemic block of Assam during 1988-1990 to ascertain impact against *An. minimus* transmitted malaria transmission in the given ecological context [23,24]. *An. minimus* has strong predilection for human host (anthropophilic index >90%) and proven vector across NE states evidenced by seasonal abundance and sporozoite infectivity for all months of the year (infection rate 3%) [25]. It is primarily an endophilic (indoor resting) and night biting mosquito actively foraging human host for blood meal with peak biting activity during midnight till early in the morning (00:00 - 04:00), thus making provision of net would prove to be an ideal intervention in relation to sleeping habits. Entomological surveys in experimental villages revealed that populations of *An. minimus* were completely decimated in intervention villages (impregnated-net users) evidenced by indoor day-resting sampling as well as human landing catches in comparison to untreated/plain net and no-net intervention villages [24]. It was imperative that pyrethroid-impregnated net served not only as physical barrier between human host and hungry vector mosquito but also prevented mosquito bites disrupting transmission. Consequently, there was significant reduction (73%) in malaria incidences in impregnated-net intervention villages over two-year period post-distribution compared to baseline year whereas rise in case incidences was observed in plain-net and no-net cluster in the corresponding study periods (Table 3). However, impact of impregnated-net intervention was assessed to be much more pronounced during low-transmission period (November-April) vis-à-vis high transmission period (May-October) in reducing *P. falciparum* case incidences (Table

4). Community responses were overwhelming for reporting decreased malaria attacks and collateral benefits of relief from other biting bugs and nuisance insects. It was clearly demonstrated that nets when impregnated with pyrethroid provided protection manifold; these were highly insecticidal with extended residual efficacy (more than six months) and are held safe for having low mammalian toxicity at given designated doses. It is a low-cost technology, easy to accomplish and held ideal for marginalized hard-to-reach population groups with poor access to healthcare services. Insecticide-treated net-based intervention (popularly known as medicated nets) was an instant success story and widely accepted. Based on the study results, Technical Advisory Committee (TAC) of the National Vector Borne Disease Control Programme (NVBDCP) decided to extend the study to other high-risk NE states to test its operational feasibility, acceptability and sustainability as an alternative technology for malaria vector control.

Table 3. Impact assessment of deltamethrin-impregnated mosquito nets on malaria transmission in a typical malaria endemic block in Assam, North-East India

Category of villages	Time period*	Study population	Malaria cases (age group) yrs.			Total cases	API/ 1000 pop**
			(<5)	(5 – 15)	(>15)		
Impregnated-mosquito net (deltamethrin @ 25mg/sq meter) ***	June 1987- May 1988	1812	61	78	205	344	190
	June 1988- May 1989	1929	44	23	139	206	107
	June 1989- May 1990	1975	18	20	62	100	51
Plain net (Untreated)	June 1987- May 1988	1500	31	64	49	144	96
	June 1988- May 1989	1788	91	98	134	323	181
	June 1989- May 1990	1874	71	68	98	237	126
No - net control	June 1987- May 1988	1630	17	25	77	119	73
	June 1988- May 1989	1823	22	67	108	197	108
	June 1989- May 1990	1850	94	158	248	500	270

*June 1987- May 1988 is the base line data, nets were distributed in May 1988;**API denotes number of cases per thousand population; ***mosquito nets were impregnated with deltamethrin @25 mg/sq meter six monthly intervals.Source Reference [23]

Table 4. Impact of deltamethrin impregnated-net intervention on malaria transmission in different seasons in a typical malaria endemic block in Assam, North-East India

Transmission season (high/low)	Incidence of malaria episodes*		Incidence ratio (Intervention/control)
	Intervention group (impregnated nets)	Control group (no-net + plain net)	
High (May–Oct. 1988)	98.90	210.71	1/2.13
Low (Nov. 1988 – April 1989)	16.31	59.58	1/3.65
High (May 1989 – Oct. 1989)	55.72	232.03	1/4.16
Low (Nov. 1989 – April 1990)	5.14	100.12	1/19.47
High (May 1990 – Oct. 1990)	51.62	97.79	1/1.89
Low (Nov. 1990 – April 1991)	14.06	58.09	1/4.13

*No. of *Plasmodium falciparum* cases per thousand population. Source Reference [23]

Given the data retrieved from three reporting states of Assam, Meghalaya and Arunachal Pradesh, it was proven unequivocally that there was marked reduction in case incidences (>50% compared to baseline year) within one year of impregnated-net intervention in the diverse ecological conditions in NE India (Table 5) [26]. Communities clearly preferred net-based intervention over residual spraying generating demands for additional supplies. What is mandated for optimal outcome is the political commitment for sustained supply of nets and insecticides ensuring periodic re-treatment exercise of community-owned nets (required to be done on six-monthly basis) and much needed compliance for community-wide protection (Figure 2).

Table 5. Impact assessment of deltamethrin-impregnated mosquito nets on malaria transmission in the north-eastern states of India*

State	Population	Time Period**	No. blood-smears examined	No. +ve for malaria parasite	% of blood-smears +ve for malaria parasite	No. of malaria cases per 1000 population
Assam	31467	Jan–Dec 1995	12713	2215	17.41	70.39
	32732	Jan–Dec 1996	2715	178	6.55	5.34
Meghalaya	8946	Jan–Dec 1995	4424	609	13.76	60.00
	10270	Jan–Dec 1996	4494	274	6.09	26.67
Arunachal Pradesh	9404	Jan–Dec 1995	6431	828	12.87	88.05
	9710	Jan–Dec 1996	5567	86	1.54	8.86

*Data collected by the respective State Health Directorate through primary healthcare services. **Data for January–December 1995 is the baseline malaria incidence. Mosquito nets treated with deltamethrin (2.5% flow) were introduced in January 1996. Source Reference [26]

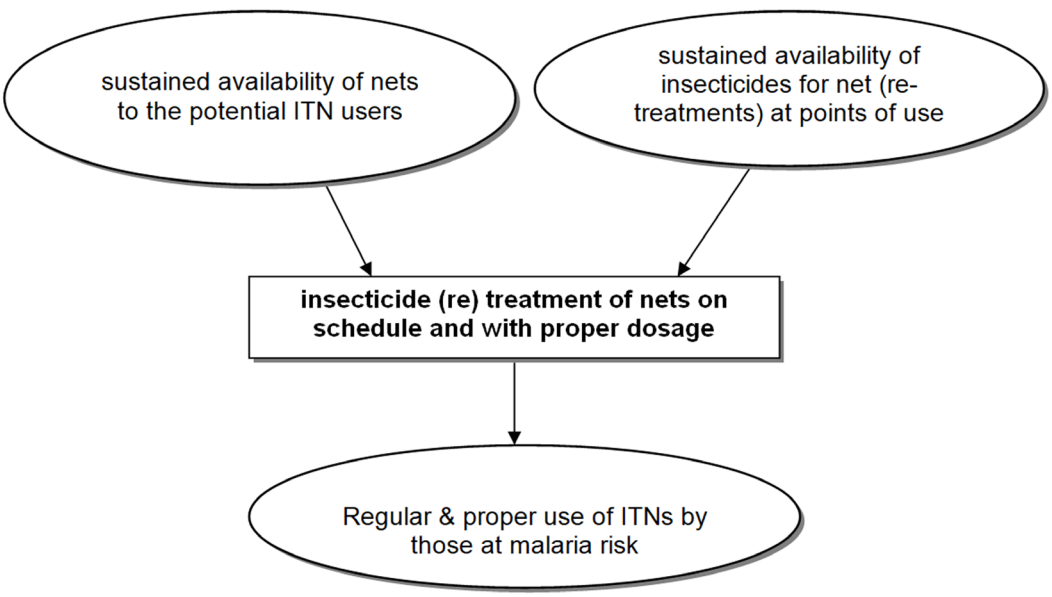

Figure 2. Flow chart of activities for sustained intervention based on insecticide-treated nets and optimal outcome for malaria control. Source Reference [26]

Long-lasting insecticidal nets: a big leap forward for vector control

Even though insecticide-impregnated mosquito nets were the choice option than indoor residual spraying by the risk-population groups, yet this technology had its own limitations on account of: (i) requiring re-treatment exercise at six-monthly intervals for waning residual efficacy, (ii) repeated washings resulted in inconsistent results for reduced bio-efficacy, (ii) manual treatment of nets amounted to erratic dosing of pyrethroid on net fiber, (iii) population coverage remained <5% of the target population. These short comings were overcome by the advent of long-lasting insecticidal nets (LLINs), a significant advancement that revolutionized the concept of vector control for added advantages over conventionally treated nets [27]. These are ready-to-use factory treated durable insecticidal mosquito nets which do not require re-treatment for 3–4 years (the net serviceable life span). Most LLINs employ pyrethroid which is either coated around polyester netting fiber (type 1) or incorporated into polyethylene polymer (type 2) before fiber extrusion. LLINs can be washed multiple times and still retain adequate residual bio-efficacy against target mosquito vector species for extended periods. Number of LLIN products have been field-evaluated globally and qualified the criterion for extended residual bio-efficacy and durability for minimum of three years of continuous use and accorded either interim or full approval by the World Health Organization [28]. Among these, Olyset net (permethrin incorporated), Interceptor net (alpha-cypermethrin coated), PermaNet (deltamethrin coated), Icon Life/Netprotect (deltamethrin incorporated), Duranet (alpha-cypermethrin incorporated) were subjected to field-evaluation for laid out criteria of extended residual efficacy, wash-resistance, durability, and impact on malaria transmission specific to NE India (Figure 3) [29-31].

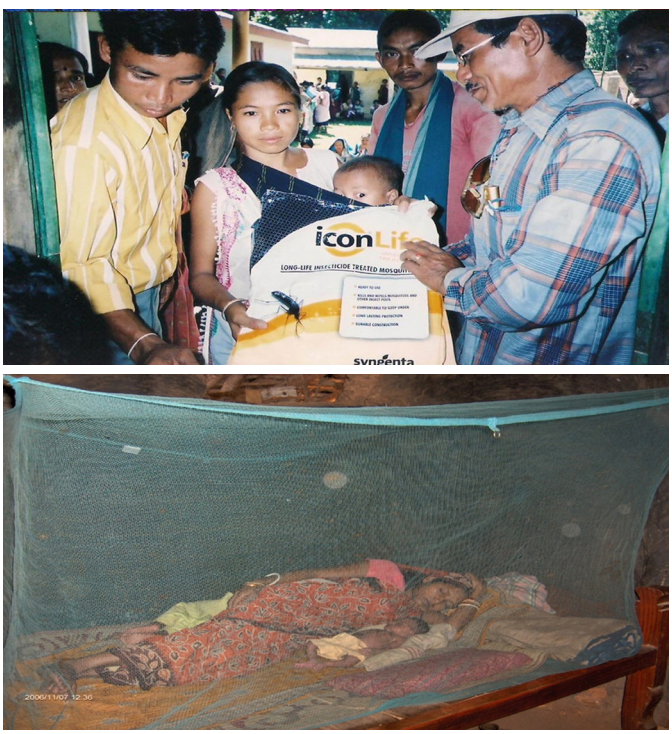

Figure 3. Top: Distribution of long-lasting insecticidal nets prioritizing vulnerable population groups in high-risk villages; Bottom: Community compliance for sleeping under net resulted in appreciable transmission reduction in the beneficiary population groups.

For data based on village scale distribution and field-evaluation of both type 1 and type 2 fiber nets, these LLINs were proven to withstand wash-resistance against 20 serial washings (Figure 4) and retaining residual bio-efficacy (≥80% kill effect against target vector species) resulting in substantial transmission reduction in beneficiary population groups (Table 6). Mass distribution of these nets in high-risk villages resulted in virtual disappearance of vector populations of *An. minimus* corroborated by data on human-bait mosquito landing catches and corresponding consistent decline in malaria incidences [32].

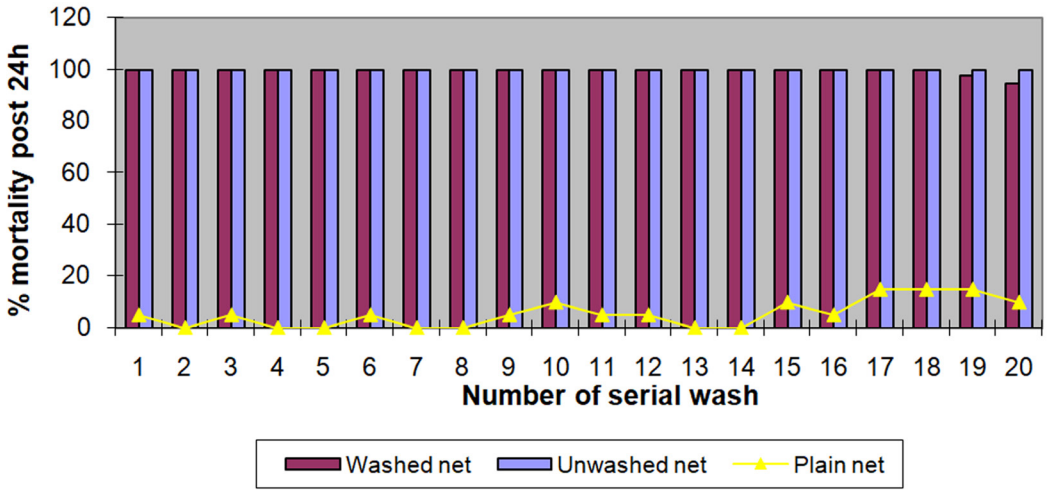

Figure 4. Wash-resistance of Icon-Life (deltamethrin incorporated) long-lasting insecticidal net expressed in terms of percent mortality of *Anopheles minimus*, mosquito vector species by cone-bioassay test method post 3-minute exposure and 24-hour recovery period in laboratory conditions subject to periodic serial washings at fortnightly intervals.

Table 6. Impact on malaria transmission of Icon-Life (deltamethrin incorporated) long-lasting insecticidalnets for data based in experimental villages of the high-risk block of Karbi Anglong district of Assam, North-East India

Category of net - intervention	Study Period*	Pop.	No. of fever cases examined	No. of malaria cases	Parasite incidence/1000 Population
Icon Life	Sept - Oct 2008	2100	635	27	12.8
	Nov 08 - June 09		2040	04	1.9
Untreated net	Sept - Oct 2008	2068	824	22	10.6
	Nov 08 - June 09		2715	51	24.6
No-net	Sept - Oct 2008	2078	342	09	4.3
	Nov 08 - June 09		2046	76	36.5

*Data for Sept - Oct 2008 is the baseline incidence; nets were distributed in October 2008.

LLINs are widely accepted and assessed to be operationally feasible community-based sustainable intervention and held appropriate technology for outreach marginalized population groups most at risk. These are becoming increasingly popular across NE communities and recommended key intervention to ward off the dreaded vectors

disrupting transmission. Number of new products are in offing encompassing combination of technologies and nets incorporating antimalarials inhibiting development of parasite in mosquito host that seem to be promising in defeating insecticide resistance [33,34].

Emerging challenges

Even though insecticide-treated netting materials have been proven efficacious against *An. minimus* and *An. baimaii* transmitted malaria (the dominant vectors in NE India), yet there are multiple challenges requiring attention of the programme and policy managers enumerated as below (Figure 5):

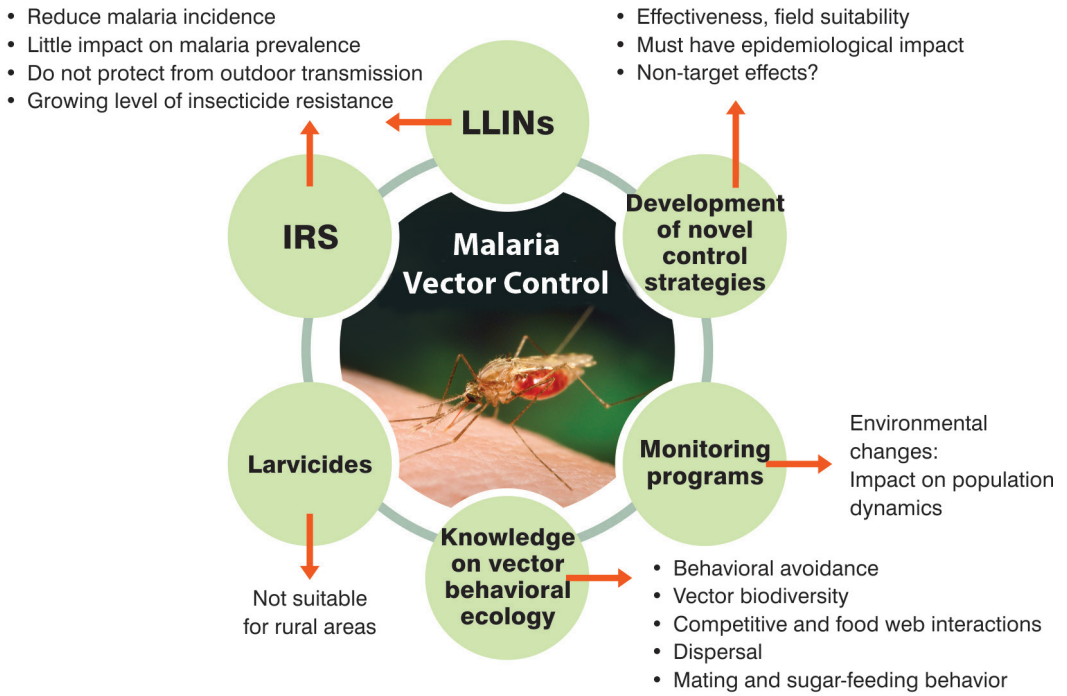

Figure 5. Vector control tools and challenges for effective vector management. Reproduced with permission. Source Reference [35]

1. Given the agroclimatic changes, populations of both *An. minimus* and *An. baimaii* are fast depleting. Instead, *An. culicifacies* (formerly occurring in low densities) is fast invading degraded forests of Assam evidenced by rising densities and incrimination records [36]. Populations of *An. culicifacies* have been assessed to be multi-resistant to the available arsenal of insecticides including pyrethroids posing renewed threat to malaria elimination initiative [17,37].
2. Requirement of LLINs is huge, yet population coverage is only miniscule of what is mandated. Additional mechanisms are warranted for increased logistic supplies and delivery services for reaching the target population making it affordable public good by reducing taxes and tariffs.

3. The net serviceable life of LLIN for its residual efficacy and durability is ascertained to be just about three years of community usage beyond which nets need to be replenished providing protection uninterrupted [32]. Currently there is no such provision for additional supplies to risk populations replacing the worn-out nets.
4. Another challenge that has emerged is the shift in vector behavior in relation to application of residual insecticides avoiding contact with treated surfaces. Vectors are getting outdoors establishing extra-domiciliary transmission – a paradigm shift that is gaining importance mandating newer tools to overcome outdoor transmission [38]. The relative risk of infection is high in forest-fringe/cross-border population/ mobile population groups for lack of awareness and poor access to healthcare access opening new possibilities of zoonotic malaria already making inroads in Southeast Asian countries [39].
5. Due to continued urbanization, other vector-borne diseases are establishing foothold in urban/suburban areas. While Japanese Encephalitis (JE) menace is increasing, dengue is one such recent phenomenon and spreading in NE India inflicting high morbidity. Indigenous transmission of dengue has been established by record of breeding of both *Aedes aegypti* and *Ae. albopictus* (vectors of dengue) and incrimination data [40-42].
6. With increased urbanization, there is high probability of invasion of *An. stephensi* in NE India (currently there is no record of its occurrence) as well with records of making home in the African continent hitherto free of this invasive species opening new vistas for control [43,44].

Taking cognizance of the issues, there is dire need for strengthening entomological capacities to address the gamut of issues including monitoring and evaluation for which expertise is getting scarce on account of attrition of skilled human resource [45-47].

Conclusions, and the way forward

North-East India is strategically significant to arrest the proliferation and spread of drug-resistant malaria for which investing heavily in vector control is of paramount importance to defeat malaria. It is amply clear that among available vector control options, LLIN is best suited intervention given the treacherous terrain and community acceptance on account of reduced risk of malaria and collateral benefits of decreased nuisance from other biting bugs. This technology is cost-effective, operationally feasible and has been duly endorsed and incorporated in the national healthcare services for vector control [48], but distribution remains patchy and far from adequate to protect risk populations. Given the added advantages of LLINs, there is window of opportunity for greater allocation of resources for scaling up distribution targeting universal coverage ensuring equitable access by means of indigenous production, social marketing, public and private partnership model (PPP), additional supplies gratis by government, donors and corporate sector alike making LLIN a household commodity to combat malaria illness. India is reporting steady decline in cases and has made impressive gains in containing transmission between 2000 - 2019 reporting 83% reduction in cases targeting malaria elimination by 2027 (49,50). Still there

is huge hidden parasite reservoir; WHO estimate many more cases which are not captured in the present surveillance and above all asymptomatic carriers which remain unattended [51]. It is the opportune time to step up efforts mounting decisive attack on disease vectors to corner malaria with rollout of evidenced-based intervention tools to prevent malaria. No single strategy may suffice rather integrated approach encompassing multiple species-specific interventions/right mix of technologies may be applied for effective vector control. The integrated vector management holds promise for not only control of malaria but also help tackle other vector-borne disease, viz., dengue, filariasis and Japanese Encephalitis (JE) endemic in NE region [52]. Community participation, intersectoral coordination, enhancing vector surveillance and monitoring, and scaling up interventions are all cardinal for sustainable control of disease vectors [53]. Each one everyone should have affordable access to healthcare services well within reach to live up with the slogan that 'zero malaria starts with me'. India stands well equipped with the present-day knowledge on disease vectors and transmission dynamics and forging ahead aiming at malaria elimination [54]; what is critical at this juncture is mobilizing resources to reach out the outreach population groups and mass movement to draw the line against malaria to achieve zero malaria status in the foreseeable future [55].

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13

Promoting larvivorous fish for biological control: fish fauna and prospects of vector control

Introduction

Vector control is an integral component of malaria containment programme which relies heavily on insecticide residual sprays (IRS) [1]. DDT is the commonly applied ever since inception of the control programme in 1953 and continues to be the choice insecticide specific to North-East (NE) India for vector containment. Even though, both malaria vector species, *Anopheles minimus* and *An. baimaii* are highly susceptible to DDT [2], malaria transmission remained uninterrupted in large tracts of land on account of variety of reasons including: (i) high refusal rates to IRS by the communities at stake, (ii) logistics for missing one of two scheduled rounds, (iii) delayed operations not coinciding with the high transmission season, (iv) lack of supervision resulting in poor quality sprays, (v) restricted access to high-risk communities/marooned villages due to incessant rains and flash floods, (vi) ethnic unrest and insurgency [3]. Besides environmental concerns, recurring costs were seen rising and returns diminishing warranting alternate interventions that are cost-effective, community-oriented, and self-sustainable [4]. Among various options, biological control employing larvivorous fish was envisaged based on demonstrated success in Gujarat and Karnataka resulting in substantial transmission reduction involving community-based approach [5,6]. Given the call by the state government, initiative was taken to explore possibility for vector control based on fish-based intervention in water bodies supporting mosquito breeding in the NE states. Included in this report is an account of faunistic surveys of indigenous fish species, laboratory assays for predatory potential and field applications for data based in Assam and its outcome enabling development of informed policy decision specific to the NE region.

Fish fauna and larvivorous potential

At the very outset, active faunistic surveys were conducted in water bodies inclusive of temporary, semi-permanent and permanent, viz., ponds, lakes, drains, streams for locally prevalent fish species. As many 36 species were identified to be occurring for data based on Kamrup district of Assam (Table 1). Many of these fish species are known to have larvivorous potential of varying degree, viz., while *Amblypharyngodon mola, Esomus danrica, Rasbora daniconius* are highly larvivorous; *Anabas testudineus, Badis badis, Chanda nama, Colisa and Puntius* species are moderately predatory; and *Danio rerio* is known to be larvivorous to some degree preferentially devouring on mosquito larvae [7]. Besides these, while *Poecilia reticulata* (guppy) was recorded to be naturally occurring in open sewage drains of Guwahati metropolis; *Gambusia affinis* (mosquito fish) was imported from state of Karnataka and mass-reared for experimental studies; both these fish species are exotic (not indigenous to India) introduced in India in 1908 and 1928, respectively, but were

advocated for mass-application for biological control of mosquito breeding due to their inherent biological characteristics [8]. Both these fish species are: (i) small in size with little food value, (ii) have high reproductive potential -grow in large numbers within short span of three months, (iii) easy to culture in local water bodies inclusive of drains/polluted waters, (iv) proven to have high larvivorous potential targeting mosquito larvae, (v) easy to transport long distances, (vi) and require no maintenance costs.While *G. affinis*, a surface feeder (top-minnow) is ideal for large freshwater bodies, viz., lakes, ponds supporting profuse breeding of *Anopheles* mosquito species; *P. reticulata* can withstand moderately polluted water bodies, viz., drains, abandoned/irrigation wells. These two species (family Poeciliidae) are remarkably similar in biological attributes (both are viviparous yielding nearly 100 young ones per brood) but can be distinguished by characteristic morphological features (Figure 1). These include body length differences and shape of teeth in jaws; guppy fish is bit smaller in length and have club shaped teeth; *Gambusia* instead is comparatively large and have sharp teeth/incisors easily discernible under dissecting microscope.

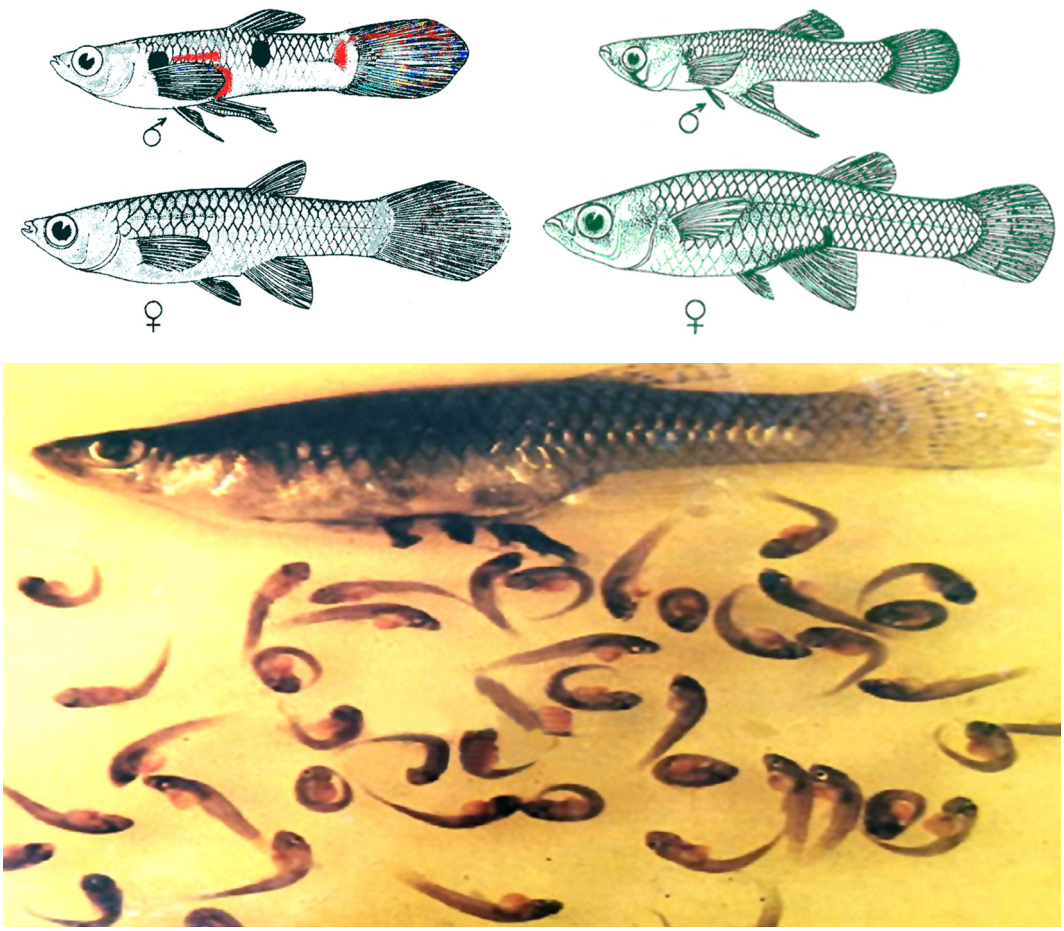

Figure 1. Larvivorous fish species, Top left: *Poecilia reticulata* (guppy fish), females ~4 cm long, ideal for drains, polluted water bodies and unused wells; Top right: *Gambusia affinis* (mosquito fish), females ~6 cm long, ideal for lakes, streams, ponds and large water reservoirs in cooler/temperate zones. Males of both species are much shorter in length (2-3 cm) and have distinct gonopodium (modified anal fin). Bottom: Both these fish species are viviparous and lay young ones/fries in broods of ~100 per gonotrophic cycle.

Table 1. Larvivorus fish fauna surveys for data based in Kamrup district of Assam, North-East India

S. No.	Fish species	Type of water body			Larvivorous potential*
		Temporary	Semi-permanent	Permanent	
1.	*Ailia coila* (Ham.)	+	-	-	-
2.	*Amblypharyngodon mola* (Ham.)	+	+	+	+++
3.	*Amphipnous cuchia* (Ham.)	-	-	+	-
4.	*Anabas testudineus* (Bloch)	+	+	+	++
5.	*Anquilla bengalensis* (G & H)	-	-	+	-
6.	*Badis badis* (Ham.)	-	+	+	++
7.	*Catla catla* (Ham.)	-	+	+	+
8.	*Channa gachua*	+	+	+	+
9.	*C. orientalis* (Sch.)	+	+	+	+
10.	*C. punctata* (Bloch)	+	+	+	+
11.	*Chanda nama* (Ham.)	+	+	+	++
12.	*C. ranga* (Ham.)	+	+	+	++
13.	*Cirrhinus mrigala* (Ham.)	-	+	+	+
14.	*Clarias batrachus* (Lin.)	-	+	+	-
15.	*Colisa fasciata* (Sch.)	+	+	+	++
16.	*C. lalia* (Ham.)	+	+	+	++
17.	*Danio rerio* (Ham.)	+	+	+	+
18.	*Esomus danrica* (Ham.)	+	+	+	+++
19.	*Glossoqobius giuris* (Ham.)	-	+	+	+
20.	*Heteropneustes fossilis* (Bloch)	-	+	+	++
21.	*Labeo calbasu* (Ham.)	-	+	+	+
22.	*L. dyocheilus* (McCl.)	-	+	+	+
23.	*L. rohita* (Ham.)	-	+	+	+
24.	*Lepidocephalus guntea* (Ham.)	-	+	+	-
25.	*Mystus seenghala* (Sykes)	-	+	+	-
26.	*M. cavasius* (Ham.)	-	+	+	-
27.	*M. vittatus* (Bloch.)	-	+	+	-
28.	*Notopterus notopterus* (Pallas)	-	+	+	+
29.	*Puntius sophore* (Ham.)	+	+	+	++
30.	*P. ticto* (Ham.)	+	+	+	++
31.	*Rasbora daniconius* (Ham.)	+	+	+	+++
32.	*Salmostoma bacaila* (Ham.)	-	+	+	+
33.	*Tetradon cutcutia* (Ham.)	-	-	+	-
34.	*Tilapia mossambica* (Peters)	-	+	+	-
35.	*Wallago attu* (B & S)	-	+	+	-
36.	*Xenontodon cancila* (Ham.)	-	+	+	-

*Based on published literature. Source Reference [7]

Comparative predatory potential of indigenous and exotic fish species

While both *P. reticulata* and *G. affinis* are widely used globally in biological control of mosquito vector populations [9], it was considered imperative to test their potential in comparison to indigenous fish species commonly found in local water bodies. In this context, predatory potential of two indigenous fish species, *Esomus danricus* (Indian flying barb) and *Danio rerio* (Zebra fish) and two exotic larvivorous fish species namely *P. reticulata* and *G. affinis* were subject to laboratory evaluation for control of mosquito breeding of common household mosquito, *Culex* species in the given ecological context [10]. It was observed that both the indigenous fish species were significantly more efficient in predating mosquito larvae than the exotic species under laboratory condition ($P<0.05$). *E. danricus* and *D. rerio* consumed 7.9±1.95 and 4.9±1.16 mosquito larvae per fish/hour in comparison to 2.40±0.17 and 2.23±0.29 by *P. reticulata* and *G. affinis*, respectively (Table 2). Among the four fish species, *E. danricus* consumed maximum number of larvae per unit time; instead, *D. rerio* consumed more larvae than *P. reticulata* and *G. affinis*, but less than *E. danricus* (Figure 2). Among these, while the predatory potential of *E. danricus* was significantly higher than all other three fish species ($P <0.05$), there was significant variation in mean predation rate among all four fish species evaluated ($P <0.001$). While *D. rerio* consumed more larvae per unit time than two exotic fish species, the difference in predatory potential of two exotic fish species was statistically insignificant ($P <0.05$). It was clearly demonstrated that both the indigenous species, i.e., *E. danricus* and *D. rerio* (Family: Cyprinidae) were more predacious than either of the exotic fish species. Both these species are abundant and widely distributed in Himalayan foothills inhabiting streams, canals, ditches, ponds, stagnant water bodies and rice fields, and should be considered for upscaling interventions as component of the integrated disease vector control strategy [11].Yet, more data are deemed necessary for field-based observations for fecundity and longevity in varied agroclimatic habitats. Nevertheless, the two exotic fish species are more advantageous for high reproductive potential and ability to withstand varied environs in diverse habitats and used universally as an integral component of the integrated vector control strategy.

Table 2. Comparative predatory potential of the indigenous fish *Esomus danricus* and *Danio rerio* and exotic larvivorous fish species, *Poecilia reticulata* and *Gambusia affinis* for control of mosquito breeding under laboratory conditions

Larvivorous Fish species	Average length of adult fish (Mean ± SD) cm	No. of mosquito larvae introduced x No. of batches	No. of mosquito larvae consumed at different time intervals per replicate (Mean ± SD)*									Mean ± SD number of larvae consumed per fish/ hour
			10 min	20 min	30 min	1h	2h	3h	4h	5h	6h	
Esomus danricus	5.50±0.25	25×10=250	14±5	26±14	28±14	39±14	74±15	117±31	153±34	197±49	237±58	7.9±2
Danio rerio	3.72±0.14	25×6=150	12±4	17±7	21±1	28±12	51±15	73±16	101±21	125±30	148±35	4.9±1
Poecilia reticulata	2.76±0.15	25×3=75	26±3	31±2	34±3	38±4	46±4	54±5	60±5	67±4	72±5	2.40±1
Gambusia affinis	2.78±0.21	25×3=75	26±5	34±5	36±5	40±6	42±7	45±9	52±8	60±7	67±9	2.23±1

*Based on five replicates each containing 5 fish adults. Source Reference [10]

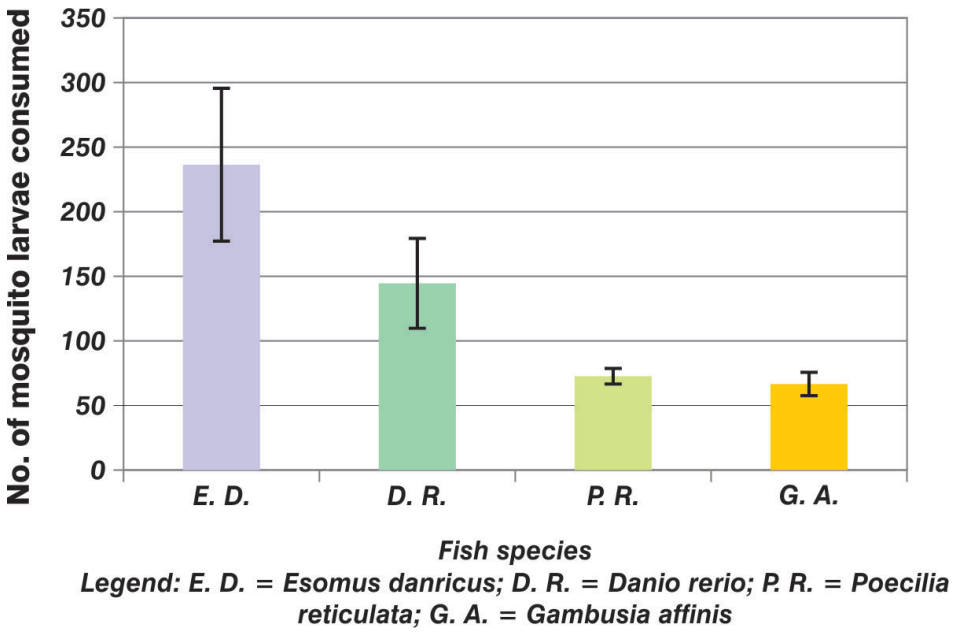

Figure 2. Number of mosquito larvae consumed by candidate larvivorous fish species in six hours' time duration. Source Reference [10]

Prospects of vector control by fish-based intervention, hope or hype?

Mass-scale application of larvivorous fish have advantages over other biological control interventions such as microbial larvicides, viz., *Bacillus sphaericus* and *B. thuringiensis thuringiensis* requiring recurring expenditures on account of repeated applications, labour costs and reports of developing resistance/reduced efficacy [12]. Instead, mosquito control using larvivorous fish is better suited being cost-effective,eco-friendly, and self-sustainable

Figure 3. The city of Sochi, Russia erected a fish statue costing about $3,800/- in honour of *Gambusia* (aka the 'mosquito fish'). In the early 1900s, the fish species were purposefully introduced to the area to fight the infestation of malarial mosquitoes, and it worked. There have been no cases of malaria among Sochi residents for over 60 years. Photo courtesy of Nadiva85/Wikimedia

Figure 4. Larvivorous fish, *Gambusia affinis* stock-pond in Guwahati City – a collaborative extension works with State Health Directorate of Assam. Number of similar water bodies, viz., cement tanks, ponds, open drainages were seeded with larvivorous fish.

community-based interventions requiring no additional costs once applied. These are self-propagating agents and proven effective intervention to check mosquito proliferation particularly in urban/suburban/town areas. There have been success stories based on fish-based intervention culminating in successful elimination of vector mosquito breeding and malaria transmission in urban ecological settings (Figure 3).

Figure 5. Oxygen packaging of larvivorous fish for long-distance transportation to high-risk districts of Assam reduced mortality to minimum.

Similarly, large scale release of *P. reticulata* (guppy fish) in wells/storage tanks and *G. affinis* (mosquito fish) in ponds, lakes/water reservoirs of sericulture districts of south Indian state of Karnataka have helped achieve control of vector mosquito breeding of *An. culicifacies* and substantial transmission reduction [13]. Preliminary surveys of presence of Guppy fish and monitoring of larval breeding sources in Guwahati City had reaffirmed efficacy of fish-based intervention resulting in overall reduction in mosquito nuisance [14]. Of 53 sites searched for presence of fish *vis-à-vis* mosquito breeding, 3 (6% of locations) had relatively low-density of fish corresponding with low-density of larval breeding (0.7 larvae/dip), 21 (40%) had high density of fish and no mosquito larvae, 23 (43%) had no fish but high larval density (72 larvae/dip), and only 6 (11%) had neither fish nor mosquito breeding. Inspired by these success stories, it was envisaged to attempt mass-produce and distribute these fish in select districts of Assam as an integral component of integrated approach in close coordination with the state/district health functionaries [15]. The work plans included establishing mother stock-ponds of both *P. reticulata and G. affinisin* state head quarters (Guwahati city) and high-risk districts involving stake holders (Figure 4). Guppy fish were used targeting mosquito breeding in polluted drains/water bodies and unused wells, and Gambusia were aimed for introduction in ponds, lakes, large water reservoirs, streams, and paddy fields. Both these fish species were transported to malaria-endemic districts for establishment of satellite stock-ponds at district headquarters for further supplies to high-risk blocks for intended release in water bodies targeting control of mosquito breeding (Figure 5).

There was clear negative association between fish and mosquito larval breeding. Similar research findings were also reported in the metropolis city of Kolkata reaffirming the observations that guppy fish can colonize and sustain in the sewage drains for long time of period helping contain mosquito populations [16]. Public perceptions were overwhelming reporting overall reduction in nuisance due to biting mosquitoes demanding further extension work not only in Assam but also other north-eastern states as well. Subsequently, satellite mother stock-ponds were established in Nagaland, Meghalaya and Manipur, and fish-stocks were also provided to public and private sector establishments alike promoting biological control of disease vectors/larval source management. While Guppy is hardy fish for surviving polluted water bodies, *Gambusia* is known to survive in colder climates; both are prolific breeder and preferentially devours on mosquito larvae evidenced by presence of larval exoskeleton/chitinous residue in droppings of fish (DK Srivastava, personal communication). Given the community acceptance, there is whole lot scope of fish-extension (that remained in oblivion post World War II) for mass-propagation and distribution helping combat mosquito menace in resource-poor settings.

Conclusions, and the way forward

One-size-does-not fit all; larvivorous fish-based intervention holds potential as component of 'integrated and inclusive vector management' that is community-driven, eco-friendly, operationally feasible, cost-savvy and befits the dogma of 'Gandhian principle of sustainable growth' [17]. Larvivorous fish culture has been successfully linked with twin benefits of mosquito control as well as edible-fish culture/income generating schemes helping elicit community involvement [18-20]. Community participation along with political

and social engagements are central to successful implementation of this intervention for disease prevention and control [21,22]. Based on this guiding principle, a framework of roles and responsibilities involving state functionaries and the beneficiaries have been field-tested in Karnataka popularly known as 'Karnataka Role Model' for optimal outcome (Figure 6).

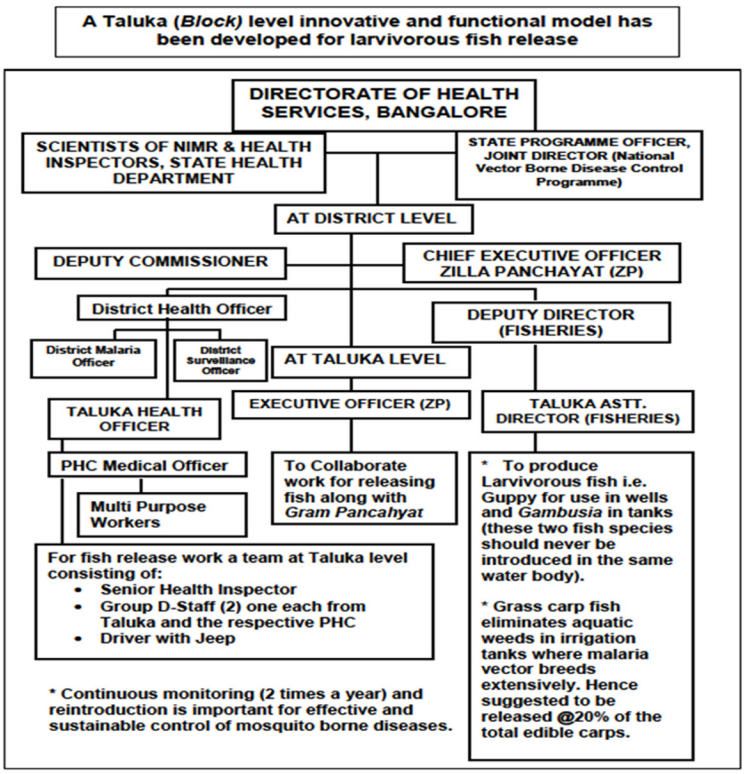

Figure 6. Karnataka role model for promoting larvivorous-fish based intervention for mosquito vector control. Source Reference [22]

Having applied this model, the state of Karnataka is reporting dramatic fall in cases with less than 2000 cases in 2020,and fast approaching pre-elimination [23]. Even though there are apprehensions for mass introduction of Guppy as well as Gambusia being exotic and invasive in nature,yet there is no credible evidence of adverse effect on local ichthyofaunal fauna in the given ecological context [24]. The application of larvivorous fish, however, outweighs the risks by multiple benefits including comprehensive vector control limiting the pathogen transmission by reducing abundance, circumventing any physiological resistance, and above all fish can sustain even in the absence of mosquito larvae [25]. Based on imminent merits, guidelines have been framed by the national control programme for upscaling distribution of larvivorous fish and monitoring for sustainable control [26].

In summary, mass-scale application of Guppy and Gambusia hold potential for mosquito population control in urban India and deserve priority minimizing risk of residual insecticides in public health [27]. Nevertheless, in conjunction with these two exotic fish

species, role of indigenous fish species should also be explored for spearheading control of disease vectors. There are number of locally abundant fish species that qualify and equally or even more voracious but remined unexploited [28-30]. Given the emerging insecticide resistance, rising operational costs and environment concerns, it is time to repurpose the use of larvivorous fish for effective vector management aligned with 'Pink Revolution' and 'Swachh Bharat Abhiyan' (Clean India Movement) for inclusive growth [31].

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Section VI

Malaria Elimination and Emerging Challenges

14

Asymptomatic malaria and treatment seeking behaviour in ethnic tribes of Assam, North-East India: implications for malaria elimination

Introduction

Malaria is a historic disease in Assam associated with recurring outbreaks and reckoned as the major public health illness [1]. Decades apart, malaria transmission continued to persist despite interventions; intensities, however, were assessed to be low-to-moderate [2]. Disease transmission and distribution is heterogenous across the landscape; parasite formula (ratio of *Plasmodium falciparum* to *P. vivax*), however, varied among districts [3].Districts with high concentration of indigenous tribes along with large forest cover sharing interstate/international border were invariably at high risk and prone to disease outbreaks [4]. Focal outbreaks were recurring and largely ascribed to sudden spurt of *P. falciparum* cases and increased morbidity marked with attributable deaths. To contain malaria, the programme largely rests on two main pillars, i.e., indoor residual spraying of insecticide against disease vectors and disease surveillance for detection and treatment of cases.Under horizontal operation post 1970's, both these measures remained off target falling short of spray coverages of risk populations and broken disease surveillance leaving many cases devoid of radical cure years together [5]. Drug therapeutic efficacy investigations were few and far in between resulting in sea of asymptomatic malaria and proliferation of drug-resistant strains [6]. Drug-resistant varieties soon became spread across North-East (NE) aided by itinerant human labour force/defence personnel and rise of *P. falciparum* malaria remained unchecked up until launch of *Plasmodium falciparum Containment Programme* (PfCP) in 1978 [7]. While disease surveillance was mandated for detection (both active and passive) and treatment of cases, asymptomatic malaria remained unattended for lack of programme policy except mass surveys in areas reporting outbreaks. There is no built-in mechanism to address asymptomatic/sub-patent parasitaemia in the programme – an issue that is gaining immense significance in the context of malaria elimination. The purpose of the present study was to ascertain the magnitude and distribution of asymptomatic (hidden sea) malaria helping formulating policy and planning to dilute the parasite reservoir to disrupt transmission. Additional objectives included study of the health seeking behaviour of risk communities, access to prevailing healthcare services and treatment practices to identify the thrust areas for strengthening health systems.

Malaria parasite burden due to asymptomatic malaria

Cross-sectional mass-blood surveys were conducted in high-risk malaria endemic districts reporting focal disease outbreaks to ascertain parasite burden and extent and magnitude of asymptomatic malaria (defined as subjects not reporting fever referred herein as afebrile) in ethnic communities. Populations investigated mostly included tribal aborigines living in low socio-economic conditions/dwelling on forest resources. Many of the villages in the districts investigated were remotely placed having poor access to healthcare services. Results obtained were exclusively based on verbal autopsy and microscopic examination

of peripheral blood-smears for malaria parasite. Malaria infections (*P. falciparum* and *P. vivax*) were recorded both in afebrile as well as febrile subjects in the districts investigated; malaria parasite rates, however, varied (Table 1). *P. falciparum* was by far the predominant infection and parasite rate ranged from 7.1–31.1% in afebrile subjects, and for *P. vivax*, it varied from 0.6–6.1% respectively. In contrast, parasite rates for either species were significantly high in febrile subjects compared to afebrile cases (P<0.0001) and ranged from 13.4– 65.8% for *P. falciparum* and 1.1–10.0% for *P. vivax* respectively. Mixed infections of both *P. falciparum* and *P. vivax* were scarce and constituted <1% of total positive cases representative of low-to-moderate transmission intensities. Variable parasite rates of both parasite species in study districts underscored the heterogenous transmission and varied parasite load across the valley.

Table 1. Malaria positivity in afebrile and febrile subjects detected in cross-sectional surveys in malaria-endemic districts of Assam, North-East India. Source Reference[8]

District	Location (Study period)	Type of collection	No. blood-smears examined	No. (%) of blood-smears	
				Positive for *Plasmodium falciparum*	Positive for *Plasmodium vivax*
Karbi Anglong	Manja (May 1991)	Afebrile	1021	73 (7.1)	8 (0.8)
		Febrile*	179	24 (13.4)	2 (1.1)
Sonitpur	Rangapara (July – Sept. 1992)	Afebrile	1321	215 (16.2)	8 (0.6)
		Febrile	38	25 (65.8)	1 (2.6)
Darrang	Tangla (July – Aug. 1992)	Afebrile	1859	258 (13.8)	114 (6.1)
		Febrile	80	27 (33.7)	8 (10.0)
Kamrup	Sonapur (Jan. – Dec. 1992)	Afebrile	4726	523 (11.1)	86 (1.8)
		Febrile	1243	311 (25.0)	117 (9.4)
Kokrajhar	Gossaigaon (July – Aug. 1991)	Afebrile	2754	858 (31.1)	54 (2.0)
		Febrile	873	386 (44.2)	23 (2.6)
Dhubri	Chappar (Sept. – Oct. 1993)	Afebrile	902	69 (7.6)	11 (1.3)
		Febrile	97	8 (8.2)	5 (5.2)

*Reporting fever at point of contact

For data based on the Sonapur Primary Health Centre (a typical foothill malaria-endemic block), an average of 14% of afebrile subjects were positive for malaria infection majority of which were due to *P. falciparum* (86%) (Table 2). Malaria cases were recorded in all months both in febrile and afebrile cases, yet infectivity and gametocyte carriage in afebrile cases was consistently high in winter season during November-December (1991–1994) for the years observed (Figure 1). Apparently, these were the residual cases not responding to standard drug-regimen in force or incomplete treatment on account of non-compliance resulting in formation of parasite reservoir inclusive of infective gametocytes resulting in continued transmission. For data based on Karbi Anglong district of Assam (hyper-endemic - known for drug-resistant malaria), it was observed that even though malaria parasitaemia were recorded in all age groups, yet the formative age (5–15 years) carried the brunt of disease burden for positivity affecting the cognitive (intellectual), social, emotional, and physical development (Table 3).

Table 2. Seasonal prevalence of afebrile parasitaemia for data based on cross-sectional surveys in the Sonapur Primary Health Centre (Dimoria Block), a typical malaria-endemic block of Kamrup district of Assam, North-East India

Month (1991-1992)	Pop. surv-eyed	Febrile cases			Afebrile cases			Total		
		No. of blood-smears examined	No. of +ve cases (%)	No. of *Plasmodium falciparum* cases (%)	No. of blood-smears examined	No. of +ve cases (%)	No. of *Plasmodium falciparum* cases (%)	No. of blood-smears examined	No. of +ve cases (%)	No. of *Plasmodium falciparum* cases (%)
Nov, 1991	889	71	37 (52)	20 (54)	236	58 (25)	48 (83)	307	95 (31)	68 (72)
December	1039	43	13 (30)	9 (69)	129	34 (26)	28 (82)	172	47 (27)	37 (79)
Jan., 1992	1523	93	30 (32)	23 (77)	316	53 (17)	43 (81)	409	83 (20)	66 (79)
February	4259	206	46 (22)	36 (78)	916	79 (9)	55 (70)	1122	125 (11)	91 (73)
March	2980	72	14 (19)	8 (57)	570	39 (7)	35 (90)	642	53 (8)	43 (81)
April	2937	101	39 (39)	27 (69)	544	76 (14)	72 (95)	645	115 (18)	99 (86)
May	2290	111	45 (40)	20 (44)	283	6 (2)	6 (100)	394	51 (13)	26 (51)
June	1882	180	58(32)	38 (66)	311	21 (7)	15 (71)	491	79 (16)	53 (67)
July	1699	135	73 (54)	62 (85)	347	116 (33)	115 (99)	482	189 (39)	177 (94)
August	1333	91	35 (38)	25 (71)	214	40 (19)	37 (92)	305	75 (25)	62 (83)
September	1614	75	22 (29)	17 (77)	267	41 (15)	36 (87)	342	63 (18)	53 (84)
October	1224	44	8 (18)	6 (75)	281	37 (13)	25 (68)	325	45 (14)	31 (69)
Total	**23669**	**1222**	**420 (34)**	**291 (69)**	**4414**	**600 (14)**	**515 (86)**	**5636**	**1020 (18)**	**806 (79)**

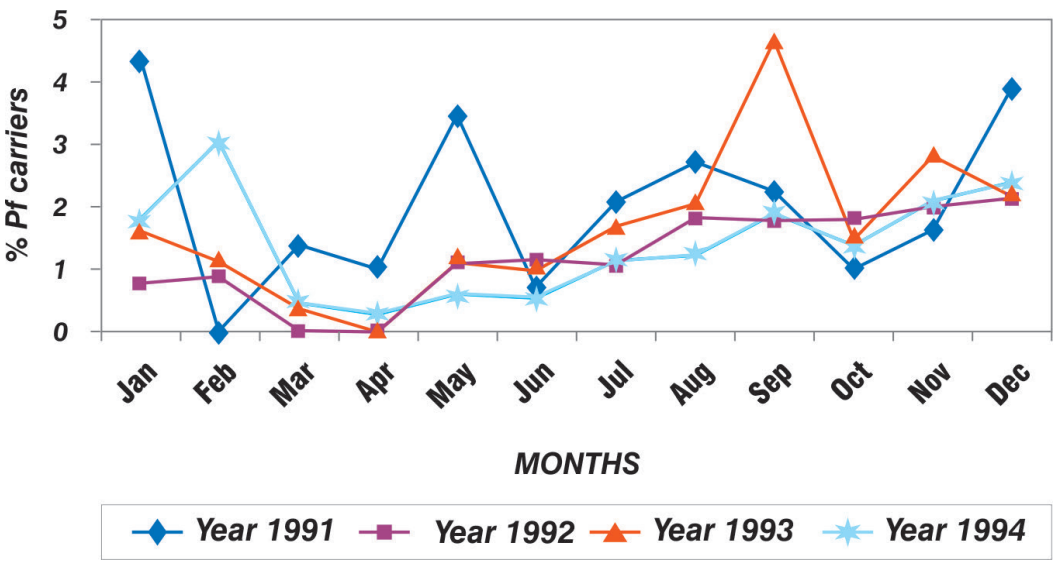

Figure 1. Monthly prevalence of *Plasmodium falciparum* carriers in the endemic communities of the Sonapur Primary Health Centre, Kamrup district of Assam, North-East India. *Source Reference [8]

While transmission intensities are ascertained to be variable across districts of Assam, asymptomatic malaria appeared to be widespread more than what was believed. Malaria was observed to be common illness in tea gardens (backbone of state economy) tribes affecting productivity on account of sickness and absenteeism. Mass blood surveys revealed that parasite rates varied across tea-estates (1.2–21.3%); however, *P. falciparum* was the predominant infection that ranged anywhere between 69-100 per cent (Table 4).

Table 3. Mass blood surveys in hyper-endemic Block Primary Health Centres(PHCs), Diphu sub-division of Karbi Anglong district of Assam, North-East India (1990-1991)

Block PHC	Pop. surveyed	Age-group in years	Febrile cases			Afebrile cases			Total		
			No. of blood-smears examined	No. of +ve cases	No. of *Plasmodium falciparum* cases	No. of blood-smears examined	No. of +ve cases	No. of *Plasmodium falciparum cases* (Pfg)	No. of blood-smears examined	No. of +ve cases	No. of *Plasmodium falciparum* cases (Pfg)
Dhansiri	350	0 - <1	0	0	0	11	0	0	11	0	0
		1 - <5	3	0	0	32	4	3	35	3	3
		5 - <15	4	1	1	43	3	3	47	4	4
		≥15	3	0	0	92	4	4	95	4	4
		Total	**10**	**1**	**1**	**178**	**11**	**10**	**188**	**12**	**11**
Manja	1278	0 - <1	3	0	0	20	2	2	23	2	2
		1 - <5	30	5	3	118	11	10 (1)	148	16	13 (1)
		5 - <15	37	5	5	198	15	10 (2)	235	20	15 (2)
		≥15	81	10	10	312	23	22	393	43	32
		Total	**151**	**20**	**18**	**648**	**51**	**44 (3)**	**799**	**71**	**62 (3)**
Bokajan	1762	0 - <1	2	1	1	8	1	1	10	2	2
		1 - <5	5	1	1	50	8	8	55	9	9
		5 - <15	8	2	2	61	7	7	69	9	9
		≥15	3	1	1	76	3	3	79	4	4
		Total	**18**	**5**	**5**	**195**	**19**	**19**	**213**	**24**	**24**

Table 4. Mass blood surveys in Tea estates of Assam, North-East India (1992-1993)

District	Tea Estate	No. of blood-smears examined for malaria parasite	No. +ve for malaria(parasite rate)	No. *Plasmodium falciparum* cases &(%) of total +ve cases
Darrang	Paneery	736	157 (21.3)	141 (90)
	Corramore	1150	235 (20.4)	141 (60)
Sonitpur	Tarajulie	1359	249 (18.3)	240 (96)
	Kolony	1392	35 (2.5)	24 (69)
	Majuligrah	165	6 (3.6)	5 (83)
	Naharani	423	5 (1.2)	5 (100)
Nagaon	Kondoli	89	17 (19.1)	12 (71)
Dhubri	Choibari	999	93 (9.3)	77 (83)
Total		**6313**	**797 (12.6)**	**645 (81)**

Community perceptions and treatment seeking behaviour

Distribution of malaria cases is highly uneven across the valley down to the household level governed by varied risk factors [2]. Communities most at stake invariably were located in forest-fringe generally away from town areas (≥5 kms) having poor connectivity for lack of public transport/motorable road [9]. The present study was based on tribal dominated block with history of reported malaria outbreaks and attributable death cases; populations

dwelled on forest resources and lived in impoverished conditions (Figure 2). Typically housing comprised of two to three rooms made of split-bamboo with thatched roofing (Figure 3). Paddy cultivation was the prime occupation for self-subsistence; others included daily wages, forest produce and handlooms. Cross-sectional surveys were conducted in these population groups during 2002–2003 for their perceptions on malaria and health seeking practices. Data were based on structured questionnaire on verbal autopsy subject to consent of participating adult members of the households. While good percentage of the populous were illiterate (53%, 461/869), some did have received primary education. Most inhabitants were aware of malarial fever but believed to be curse of the almighty and were just as reluctant to have blood-smear examined for malaria parasite until it turned pernicious resulting in belated treatment. Nevertheless, while 89% (773/869) of inhabitant interviewed opted for government facility for treatment being free of costs, 7% sought treatment form private practitioners generally located in town areas, 3% approached pharmacies and<1% opted for self-medication. The time gap between onset of fever and self-reporting ranged from 1 day (9%), 2 days (31%), 3 days (14%) and 4 days (46%). Most inhabitants walked to nearby healthcare facility (35%), while others used bicycle (22%), or hired auto/taxi service for seeking treatment. Among those reporting fever, only 40% had their blood-smear examined, and remainder received symptomatic treatment.

Figure 2. Indigenous tribal folks in their natural attire little aware of malaria prevention and control were vulnerable to malarial infection. Tribal communities rich in their heritage have their own dialects and cultural practices.

Of various treatment options, majority (63%) opted for oral therapy rather than parenteral formulations and had spent ranging from equivalent of one US dollar to 90 dollars per episode. Virtually most households used mosquito net (99.6%) but had strong reservations for their premises to be sprayed with DDT (the commonly used insecticide for vector control). Of the respondents, only 65% permitted indoor residual sprays, others denied on account of being inconvenient (69%), making the house look dirty (30%), foul smell (25%), and many did not believe that DDT is effective (13%), fear of adverse reactions, viz., headache, sneezing (3%); other reasons included having the house locked and worse

Figure 3. Typical housing structure made of split-bamboos and thatched roofing. House interiors and exteriors were routinely mud-plastered on festive occasions coinciding with the high transmission period. Most households owned mosquito nets for personal protection against mosquito nuisance; additional nets were provided gratis to overcome short supply for family protection.

some believed that nuisance increased due to biting bugs, and for rearing silkworms and honey bees etc. Many of the respondents (19%) were noted to have their household mud-plastered just after residual spraying operations rendering it ineffective.

Healthcare services and practices

Besides disease surveillance and treatment of cases derived from both the sources (active and passive case detection), two rounds of DDT residual sprays @ 1 gm per square metre are scheduled in the control programme to contain malaria. While active surveillance based on fortnightly domiciliary visits remained inadequate on account of variety of operational reasons, viz., villages remained marooned on account of flash-floods/incessant rains for days/weeks together, houses found locked; residual sprayings was far from satisfactory for falling short of adequate spray coverage of targeted populations, untimely sprays, missing rounds, high refusal rates for not permitting indoors, poor quality operations for lack of intermediate supervision. For data based on community surveys conducted during 2002–2003, as many 64% (326/510) denied visit of any health worker to their premises. Often a times, there remained wide gap that ranged anywhere from 2 weeks (2%), 3 weeks (1%), and 4 weeks (4%) between blood-smear collection and administration of radical treatment of positive cases resulting in extended morbidity. Worse come, majority (62%, 316/510) of the respondents admitted for not taking radical treatment. Consequently, large sections of high-risk communities remained devoid of interventions resulting in persistent transmission. Continuous neglect years together resulted in sea of parasite reservoir that remined untreated resulting in 'herd immunity'. Surveys among practitioners (N=57) revealed that malaria was the major public health ailment among those reporting fever. Most physicians preferred chloroquine as first line of treatment but reported drug-failure to the extent of 30% of subjects prescribed; alternate drugs opted

included quinine (77%) and/or artemisinin derivatives depending on clinical presentation. As a matter of their records, subjects presenting severe complications had wide array of clinical symptoms including cerebral involvement, anaemia, hypoglycaemia, and altered sensorium/convulsions, most of which survived the ordeal (98%). Most practitioners (>90%) performed supporting diagnostic tests including examination of blood picture, liver functions, and sugar estimation. Common chemotherapeutic options included artemisinin derivatives, viz., artesunate (86%), artemether and/or arteether (9% each) as curative therapy invariably coupled with supportive therapy such as glucose saline, paracetamol, antibiotics, anti-convulsant and steroids depending on clinical presentation. Reasons for malaria mortality included late reporting (91%), hepatic jaundice (64%), mis-diagnosis (45%), non-compliance and improper treatment (32% each). Most prescribed anti-malarial medicines (marked free supply under primary health care) were also readily available in the market but healthcare and diagnostic services were invariably centred in town areas having secondary and tertiary care facilities.

Constraints and implications in malaria elimination

Active surveillance has become a distant reality in population spread as massive as in India to ensure complete coverage and treatment compliance. Annual blood examination rate has invariably fallen short of the target coverage of 10% (expected fever rate in the communities of all causes) specific to NE region of India on account of myriad of reasons [10]. Repeated malarial attacks and incomplete treatment in space and time had resulted in huge parasite reservoir and herd immunity in endemic communities presenting varied clinical presentation. Asymptomatic malaria was recorded to be widespread in the NE, but proportion of carriers varied in relation to endemicity and propensities of transmission [8,11,12]. In fact, asymptomatic malaria is widespread across the country than considered and would be the one major constraint which need to be addressed to defeat malaria [13-19]. Subjects with mild clinical presentation (more so in far-flung tribal communities) often do not seek medical care and serve as silent carriers for persistent transmission of the causative parasites. Specific to NE region, high endemic pockets with predominance of *P. falciparum* had greater concentration of carriers (>30%), instead in low-to-moderate transmission areas, 8-20% subjects were asymptomatic with parasitaemia (Table 1). Yet, this could be only tip of iceberg as proportions of subjects with sub-patent/low-density parasitaemia remained unearthed. It is estimated that nearly 50% of infections are missed by conventional methods in vogue inclusive of both microscopic examination of peripheral blood-smears as well as rapid diagnostic test kits [20]. Application of PCR – based technologies have revealed positivity for malaria parasite manifold, which were apparently missed, thus parasite burden is much greater than reported. Nevertheless, with the advent of newer interventional technologies (artemisinin-based combination therapies in particular), India has made laudable progress in achieving appreciable transmission reduction and fast accelerating towards the goal of malaria elimination by 2027 [21]. As the cases become scarce, priority must be established for detection and treatment of asymptomatic cases as well as those with sub-patent/low-density parasitaemia by strengthening diagnosis by technologies which are affordable and sustainable [22-24]. Malaria elimination would be unthinkable unless problem of asymptomatic/sub-patent malaria is addressed in its entirety to realize the goal of ending transmission in the foreseeable future.

Conclusions and specific recommendations

It is amply clear that asymptomatic malaria is widely prevalent and needs attention of programme and policy managers to dilute the parasite reservoir [25]. Malaria burden is huge than what is being projected having implications in elimination efforts [26]. Additional surveillance mechanisms are warranted reaching out the outreach population groups ensuring radical cure [27]. India holds the capacity given the blueprint to contain COVID-19 onslaught which can be put to practice to mitigate the malarial threat [28]. Programme should be oriented to delimit high-risk pockets and prepare logistically to launch massive hunt ensuring radical treatment disrupting transmission. High-risk areas having history of malarial outbreaks should be prioritized for greater allocation of resources for universal coverage of interventions uninterrupted. Healthcare services should be strengthened upgrading skills of technical staffs with modern diagnostics to test-treat-track cases helping minimize the malarial threat [29]. In this context, while rapid diagnostic kits (RDKs) have proven handy tool for on-the-spot test result at doorstep, artemisinin-based combination therapies (ACTs) have done the magic trick in containing spread of drug-resistant malaria. Much more can be achieved by accomplishing the pending task of targeting asymptomatic reservoir with newer interventions that are robust, doable, and sustainable. While there is a paradigm shift in vector behaviour [30], parasite is also continually evolving against available arsenal of antimalarials posing renewed threat to the malaria elimination efforts [31]. Monitoring and evaluation should be the cardinal activities for decisive action against both the culprit vector mosquito and the clever parasite. Communities at stake should also shoulder the responsibility in providing compliance for call to action for successful outcome. Universal health coverage coupled with increased awareness and prevention hold the key for which stakeholders should join hands in minding the funding gap by enhanced provisions to meet the logistics uninterrupted – together we can draw a line against malaria.

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15

Climate change and malaria transmission in Assam, North-East India: retrospective analysis

Introduction

North-East (NE) India is rich tropical bio-sphere and receives heavy rainfall by southwest monsoons supporting diverse fauna and flora. Mosquito fauna is rich and breeding habitats and diverse and numerous supporting multiple vector species transmitting mosquito-borne diseases like malaria, lymphatic filariasis, Japanese encephalitis and dengue taking heavy toll on account of morbidity and attributable deaths [1]. Malaria is common febrile illness, disease transmission intensities, however, are heterogenous across the region affecting all age groups across gender [2]. Climatic conditions are conducive almost throughout the year for mosquito proliferation and longevity supporting heavy to moderate transmission. During the past few decades, NE has witnessed a vast transformation on account of expanding infrastructure notably the highway road network, industrialization, urbanization, increased agricultural acreage/land use pattern and population influx at the expense of large-scale deforestation having implications on climatic and ecological changes [3]. Malaria epidemiology is governed by host of determinants of which temperature is critical for development of parasite in the mosquito host (sporogony) [4]. Seasonal rise in temperatures associated with anthropogenic activities could amount to increased entomological inoculation rate on account of the following factors: (i) reduced duration of sporogony of parasite in the mosquito host, (ii) shorter mosquito life cycle/high reproduction rate, (iii) increased mosquito biting intensities escalating malaria transmission particularly *Plasmodium falciparum* (the killer parasite). With climate change high on the agenda, it was considered prudent to review malaria data in the context of meteorological factors retrospectively to ascertain emerging trends (if any) helping devise control strategies mitigating malarial threat. Included in this review are epidemiological data of the preceding years (1986-2009) in relation to rainfall pattern, temperatures, and mosquito vector density to have informed policy in place for benefit of the control programme.

Rainfall and seasonal transmission

NE receives heavy downpour lasting almost six-months commencing April till September and the total rainfall varies between places ranging from 2-3 metres/year in the valleys except few locations up in the hills with record precipitations (~10 metres). Active malaria transmission commences with the onset of pre-monsoon showers in April and the seasonal peak (April - September) corresponded well with the months of rainfall up until its cessation in October beyond which transmission continues but in low intensities. This was also the high transmission period and pattern was consistent across years, yet transmission intensities varied between years and places (Figure 1). The sudden rise in cases was solely due to *P. falciparum* which collectively remained the predominant infection in the NE region, the remaining were *P. vivax* cases.

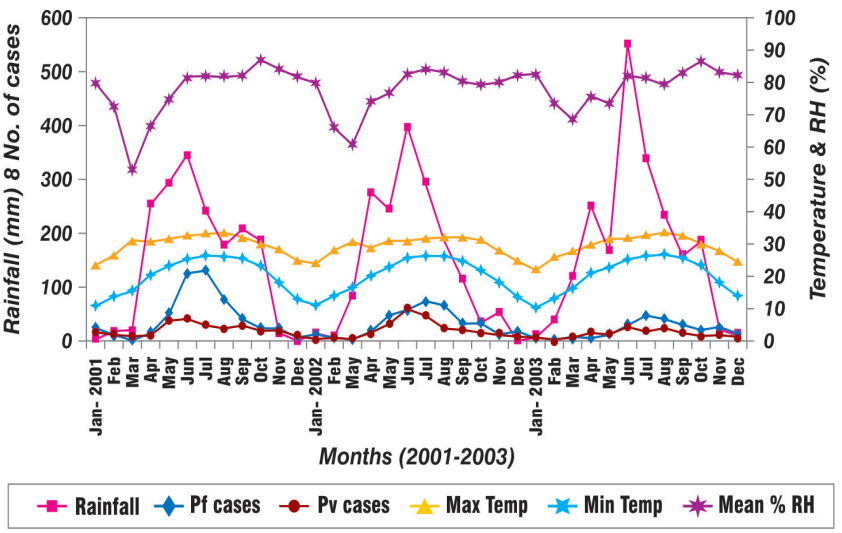

Figure 1. Monthly distribution of malaria cases in relation to meteorological indicators in the Sonapur Primary Health Center (Dimoria Block), Kamrup district, Assam, North-East India. Source Reference [5]

There was significant fall in *P. falciparum* cases during October - March (dry-season), the transmission of *P. vivax*, however, continued uninterrupted throughout the year with little variation. Taken together for data on annualized rainfall and annual malaria incidences (number of cases per thousand population) for the period (1986-2009) based on typical malaria-endemic block in Assam, it was observed that correlation between these two variables was too weak and statistically insignificant (r = 0.070; df = 22; P = 0.750). During these years, malaria incidence decreased substantially 1998 onwards irrespective of rainfall, instead the unprecedented rise in cases during 1994-1997 was due to focal outbreaks (Figure 2).

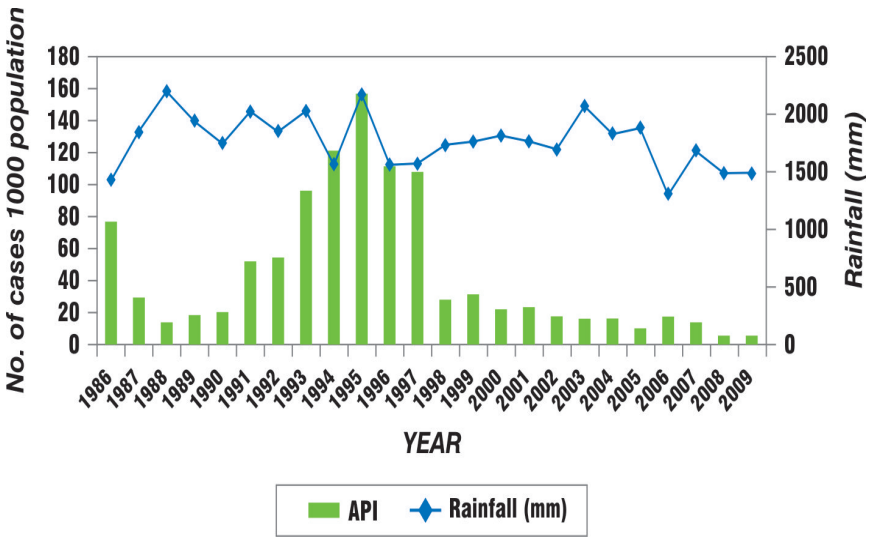

Figure 2. Rainfall and annual parasite incidence (API = no. of cases per thousand population) for data based in the Sonapur Primary Health Centre (Dimoria Block), Kamrup district, Assam, North-East India (1986-2009). Source Reference [5]

Conversely, while the rainfall was the highest during 1988, malaria incidence was much lower in the corresponding year. Further more, correlations between annual rainfall and number of malaria cases derived from passive surveillance (r = 0.200; df = 17; P = 0.411) and that of annual number of rainy days and malaria cases (r = 0.223; df = 17; P = 0.359) were also insignificant (Figure 3). The relative humidity (%RH), however, was consistent for years considered (>69%), but there was no apparent correlation with case incidences (r = 0.087; df = 17; P = 0.723).

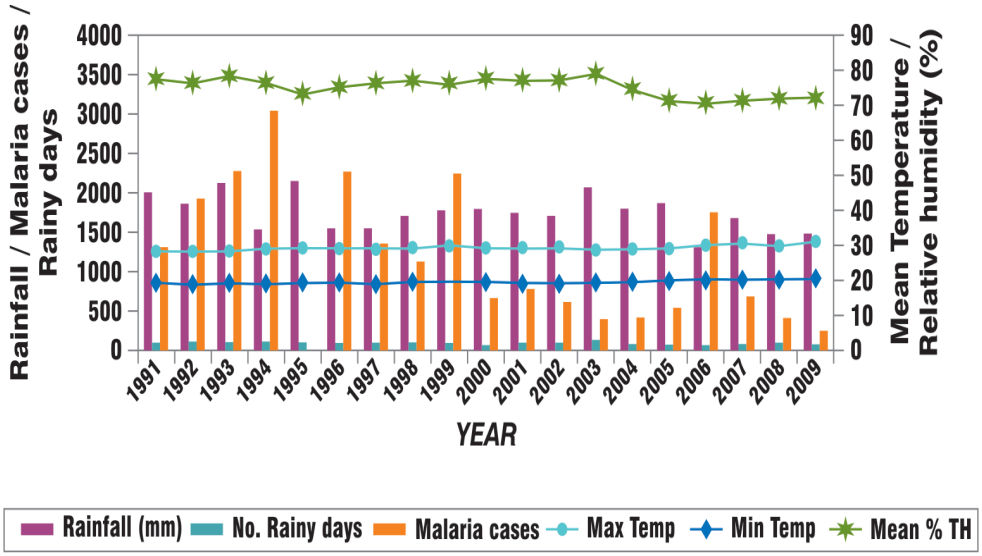

Figure 3. Mean annual rainfall, number of rainy days, annual maximum and minimum temperature (^{0}C), annual mean relative humidity (%), and malaria cases for data based in the Sonapur Primary Health Centre (Dimoria Block), Kamrup district, Assam, North-East India (1991-2009). Source Reference [5]

Temperature and malaria

Temperature plays a critical role in the development of malaria parasite in the mosquito host as well as survival of vector maintaining density well above the threshold level for active transmission of *Plasmodium*, the causative parasite. It was observed that during high transmission season (April – September), the difference between maximum (29-33^{0}C) and minimum (20-26^{0}C) temperatures was uniform and less marked vis-à-vis remainder of the year (Figure 1). It is hypothesized that sudden rise in *P. falciparum* cases beginning April was due to congenial temperature ranges that helped rapid development of the parasite in the vector mosquito host resulting in preponderance of cases. Instead, fall in minimum temperatures amounting to wider range commencing onset of winter season beginning October arrested the development *P. falciparum* to some extent without interrupting *P. vivax* transmission. It was observed that seasonality of malaria transmission particularly *P. falciparum* was significantly correlated with mean temperatures (r = 0.990; P = 0.010) for which rise in maximum temperatures was consequential (r = 0.997; P = 0.003). However, correlation between annualized mean maximum temperature and malaria cases for the years (1991-2009) was insignificant (r = -0.126; df = 17; P = 0.607).

Meteorological factors and vector density

Malaria transmission involves complex interplay between both extrinsic and intrinsic factors of which vector mosquito is an essential component of the chain to complete the sporogony cycle of the malaria parasite (Figure 4). Seasonal abundance, survival and longevity of mosquito vector is governed by favourable climatic determinants of which temperature is a detrimental factor for maintaining critical density to support active transmission. Among anopheline mosquito species indigenous to NE, *Anopheles minimus* and *An. baimaii* were unequivocally established efficient vector species transmitting malarial parasite by records of incrimination in their domains of distribution [6].

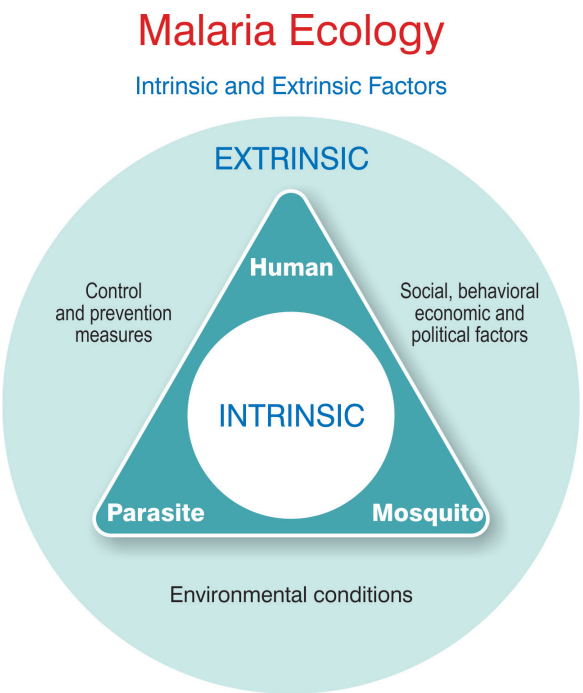

Figure 4. Malaria ecosystem: intrinsic and extrinsic factors governing malaria transmission involving human, parasite, and the mosquito. Courtesy: V.P. Sharma

An. minimus mosquitoes were recorded throughout the year, however, their density (number of mosquitoes per person hour) started rising from March onwards with the onset of rainy season/pre-monsoon showers and was the highest during April-September corresponding to months of rainfall (Table 1) [5]. These were also the months when mean temperatures were the optimum and mean relative humidity (>80%) was the highest.

For remainder of the year, vector density was comparatively low corresponding with months of low rainfall and decreasing temperatures (Figure 5). However, the build-up of vector density preceded that of high transmission season that commenced from April onwards owing to the extrinsic development of parasite in vector mosquitoes as well as incubation period in the human host. However, correlations between mosquito vector density and rainfall ($r = 0.605$; $df = 5$; $P = 0.150$) and that of mean temperatures and rainfall ($r = 0.558$; $df = 5$; $P = 0.193$) were statistically insignificant.

Climatic determinants and spatio-temporal distribution of malaria

Most districts of NE region are malaria-endemic and contribute to substantial number of cases annually, yet relative risk of malaria varied in the given ecological context in space and time. Focal outbreaks were of common occurrence few years apart characterized by sudden spurt of cases and attributable deaths. Rainfall pattern and forest cover also varied between places, viz., districts of upper Assam received substantially higher amount of precipitation than that of lower Assam. In 2002, several districts of Assam namely Golaghat, Dhemaji, Darrang, Lakhimpur, Sonitpur, Nalbari, Tinsukia and Nagaon reported high morbidity, but correlation between rainfall and malaria data was too far weak ($r = 0.024$; $df = 6$; $P = 0.980$) and insignificant (Figure 6). Likewise for most districts of Assam reporting high rise in cases (data not shown) in 2006, correlation between rainfall and malaria incidences was also insignificant ($r = -0.054$; $df = 20$, $P = 0.811$).

Table 1. Seasonal mosquito vector density and meteorological indicators for data based in the Sonapur Primary Health Centre (Dimoria Block), Kamrup district, Assam, North-East India

Study period (1989-1991)	Total Rainfall (mm)	Mean Relative Humidity (%)	Mean mosquito vector density*	Average maximum temperature (^{0}C)	Average minimum temperature (^{0}C)	Mean Relative humidity (%)
Oct - Dec 1989	120	81.66	1.34	26.76	16.76	82
Jan - Mar 1990	109.4	72.3	6.18	25.53	13.86	73
Apr - Jun	641	78.3	15.09	30.2	22.66	79
Jul - Sep	855	82	6.85	31.96	25.43	83
Oct - Dec	145.4	82.6	2.17	27.7	17.36	83
Jan - Mar, 1991	81.9	69.3	3.59	26.93	13.63	70
Apr - Jun	863.3	77	7.22	29.86	22.43	76

*Number of *Anopheles minimus* mosquitoes collected per person hour in the human dwellings (indoors)

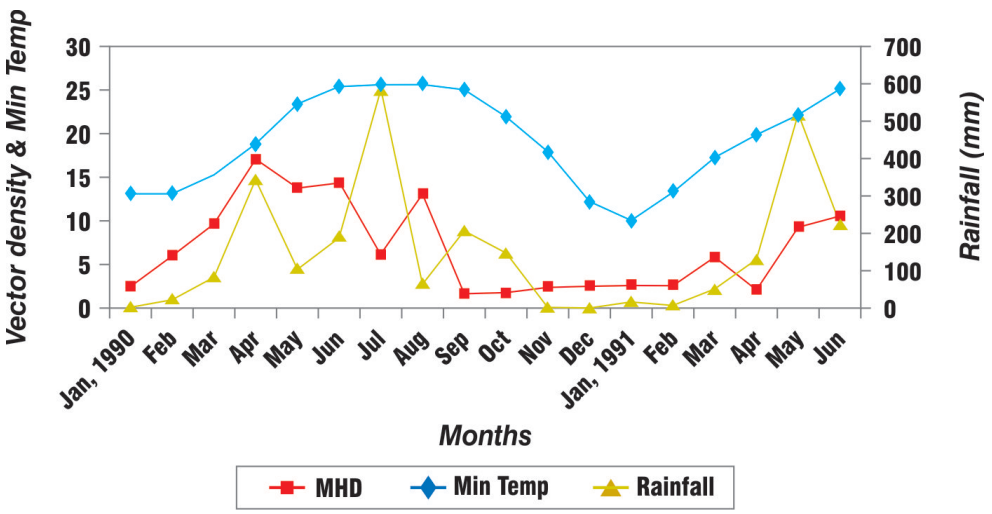

Figure 5. Monthly rainfall, mean minimum temperature (^{0}C) and density of vector populations of *Anopheles minimus* (MHD) for data based in the Sonapur Primary Health Centre (Dimoria Block), Kamrup district, Assam, North-East India (1990-1991)

Climate change and emerging transmission trends

Global warming is associated with increased anthropogenic activities and associated carbon emissions. Spatial pattern of trends in mean annual temperature anomalies revealed significant positive (increasing) trend over most parts of the country except over some parts of Rajasthan, Gujarat and Bihar, where significant negative (decreasing) trends were observed (Figure 7). NE India is no exception for witnessing exponential transformation. Even though rainfall pattern varied between years, yet there has been a measurable increase in temperatures associated with El Niño Southern Oscillation (ENSO) specific to NE region. Among the years considered, 2009 was recorded to be warmest ever since 1901 with annual mean temperature of 25.55^{0}C (+0.913^{0}C higher than long-term national average between 1961- 1990). Since 1901 of the 12 hottest years, eight were just in the past decade alone (S. Das, India Meteorological Department, personal Communication). Furthermore, mean monthly minimum and maximum temperatures were recorded to be the highest in January, August, and September in the past five years (2005-2009). The rise in temperatures is poised to increase transmission season and spread of vector-borne diseases to areas hitherto considered to be low-risk.

However, in the present study given the interventions in-force in the NE region, nothing could be ascribed to climatic change. There was no significant association for long term data between absolute rainfall and inter-annual variation in malaria cases. Similarly, variation in mean temperatures did not account for variable transmission intensities observed between the years. Instead, disease transmission trends were clearly deaccelerating over the years observed commencing turn of the century. Similar observations were reported in Central India (having large forest cover and heavy downpour during monsoons) reporting persistent disease transmission with no such association between malaria incidences and climatic variables [7]. Seasonal transmission, however, particularly that of *P. falciparum*

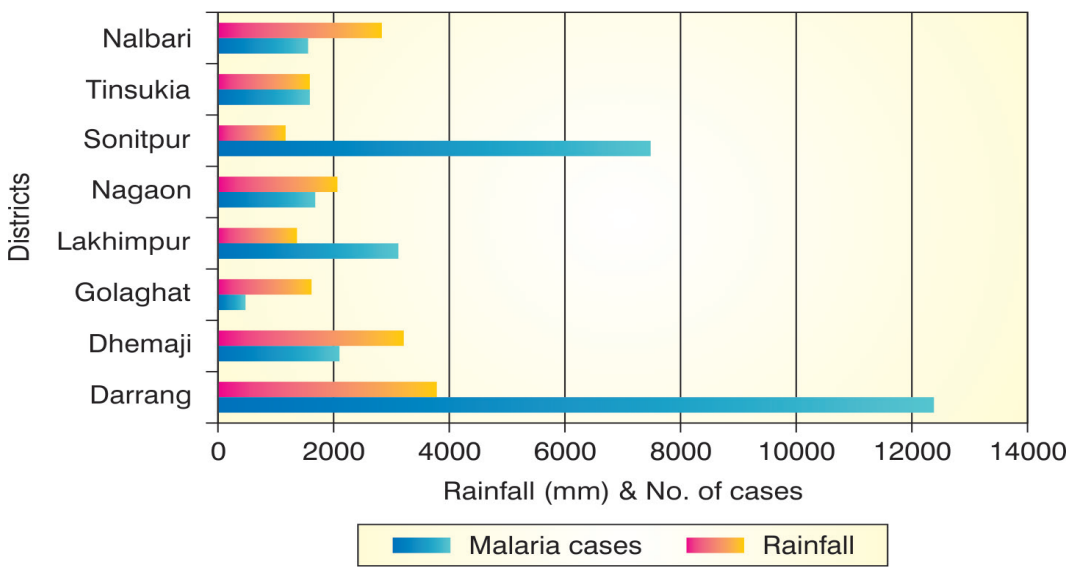

Figure 6. Annual rainfall and malaria cases in districts of Assam reporting malaria outbreaks in 2002 (Data source: State Health Directorate of Assam). Source Reference [5]

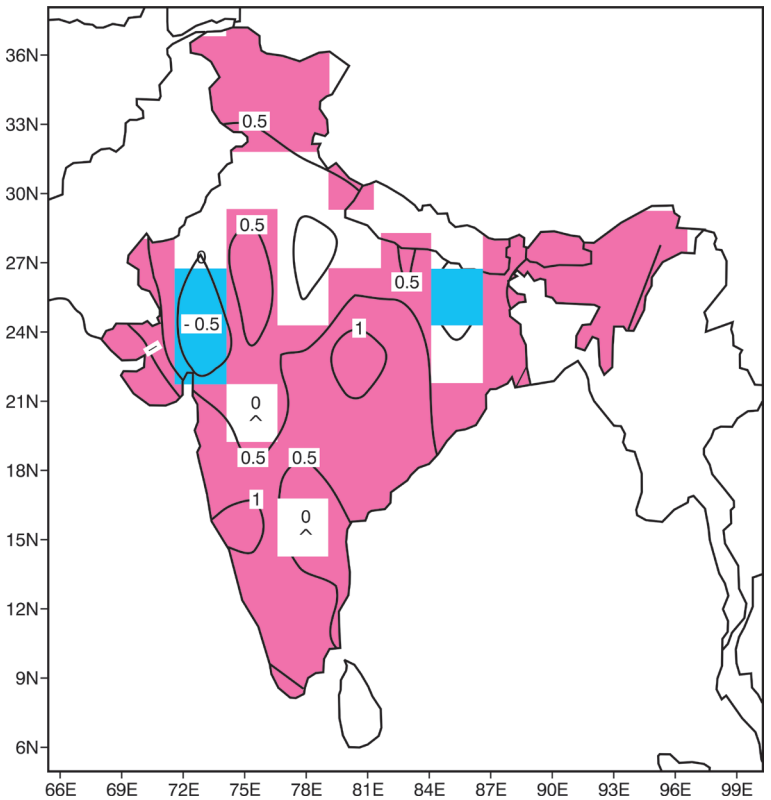

Figure 7. Spatial pattern of increasing trends in mean annual temperatures in India (1901 – 2009). Red zones denote increase in temperature by given digree across Indian landscape. Source: S. Das, India Meteorological Centre, Guwahati.

was positively related to meteorological indicators, viz., onset of rains early on, rise in temperatures; something to look out for, understanding of which could help developing dynamic model of prediction giving lead times for contingent plan of action mitigating imminent disease outbreaks [8,9]. The mean temperatures, however, are poised to increase globally in the coming decades which could amount to increased transmission window particularly spread of *P. falciparum* malaria replacing *P. vivax* transmission in high-land areas [10]. NE contributes bulk of *P. falciparum* cases (>90%) and remains the majority infection in NE states of Tripura, Meghalaya, and Mizoram [2]. These are also the states reporting focal disease outbreaks related to emerging multi-drug resistant strains amounting to insurmountable disease burden beyond reach of the healthcare system [11,12]. In these states' morbidity is alarmingly high associated with the uninterrupted transmission perpetuating vicious cycle of malaria and poverty. The relative risk of malaria is high in these states for sharing long international border and poorly coordinated vector control operations, and lack of awareness on disease prevention and weaker healthcare access.

Conclusions

North-East India is a tropical wetland ecosystem and malaria has been the major public health concern for recurring focal outbreaks and colossal operational costs. Nevertheless,

with the added substantive knowledge on disease vectors and transmission dynamics [13], there is a renewed optimism for freedom from malaria. Malarial threat is receding with the rollout of present-day intervention tools including efficacious antimalarial drugs (artemisinin-based combination therapy) and strengthening vector control options providing coverage with insecticide-treated nets/long-lasting insecticidal nets preventing mosquito bites [2]. There is no shred of evidence on record for increased malaria receptivity in the changing climatic scenario and ecological context. Given the interventions in force, all projected models/forecasts are belied for increased window of transmission [14-16], rather trends are clearly downwards each passing year fast approaching pre-elimination at the sub-national level [2]. On the contrary, disease is reportedly expanding in the dry-land ecosystem (north-western Indian state of Rajasthan) in the changing agroclimatic context in consonance with projected models for increased *P. falciparum* case incidences [17,18]. Malaria is an interventional disease failing which transmission can upsurge in the tropics putting more population at risk given the conducive climate and ecology resulting in disease outbreaks and spread of malaria [19]. The continuing transmission in Tripura, Mizoram and Meghalaya rather is an operational failure and attributed to inadequate interventions on account of fragmented disease surveillance, logistics and poor health systems in the periphery. It is apparent that rainfall data alone are not sufficient predictor for early warning for impending malaria outbreaks in the wetland ecosystem [20]; more robust models are warranted for accurate predictions taking into account seasonal dynamics [21]. Instead, it is more that relates to human activities (non-climatic factors) and local physiographic risk factors that should be considered providing lead times for improved resource allocation to avert impending disease outbreaks [22]. Similar conclusions were drawn in East Africa reporting lack of association between long-term meteorological trends and local malaria resurgences [23]. In the similar vein, while malaria map is shrinking with many countries certified malaria-free [24], India is also forging ahead with many states reporting less than 1000 cases and fast reaching its goal to end malaria transmission by 2027 [25]. Complacency at this stage would cost heavily with resurgence in vengeance not only permitting re-establishment of malaria transmission in malaria-free territories but also spreading to non-endemic areas [26]. What is tantamount to defeat malaria is building stronger health systems for increased awareness and disease prevention, focused interventions, sustained logistic supplies and capacity building for delivery in time and place by improved surveillance and healthcare access for those most at risk [27]. Set aside malaria, there are already signs of ecological shift towards heightened risk of transmission of arboviruses such as dengue [28,29], Japanese Encephalitis (JE)/ Acute Encephalitis Syndrome (AES) calling for robust heath systems to meet the emerging paradigms [30].

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16

Behaviour change communication: health education and human resource development

Introduction

Malaria transmission in India is complex on account of its varied ecology, disease distribution and determinants [1,2]. Transmission intensities are heterogeneous with large concentration of cases in forested areas of the eastern, central, and north-eastern states contributing over 80% of reported cases and deaths [3,4]. Majority of these cases are due to drug-resistant *Plasmodium falciparum* associated with high morbidity and attributable deaths. These are also the areas with strong presence of indigenous tribes thriving on forest produce and distinct practices characterized by housing conditions, regional dialects, and costumes. Despite decades of attempted control, disease transmission in these areas remained uninterrupted on account of low socio-economic conditions and abundance of efficient vectors. The relative risk of malaria is ascertained to be high in these pockets for lack of communication means, awareness on disease prevention and access to treatment [5,6]. Majority populous in forested belts generally lived in poverty largely dependent on forest economy and meagre wages. Poor housing conditions and location adjacent to vector breeding habitat resulted in increased human-vector mosquito contact/entomological inoculation rates amounting to heavy transmission [7,8]. Healthcare facilities invariably were distant leaving populations devoid of disease surveillance and treatment. Access to these pockets was often beyond reach due to lack of road communication, marooned by incessant rains/flash floods leaving them aloof of interventions particularly during high transmission season. Most affected population groups were invariably illiterate and just as ignorant for seeking treatment from the nearest healthcare facility rather opted for services of unqualified physicians (quacks) or self-care and even worse remained untreated [9]. Many households were just as reluctant to allow access to their premises and clearly opted out for confirmed diagnosis of malaria by blood-smear examination; compliance to radical treatment schedule was only a distant reality. Inadequate interventions years together resulted in building up of vector density and intense transmission amounting to high morbidity and creation of asymptomatic reservoirs, the root-cause of persistent transmission. North-East (NE) India has huge forest cover (>40% of land area) and home to host of indigenous tribes living in forest-fringe areas reporting focal disease outbreaks with poor access to healthcare facilities. These communities little aware of disease prevention and control bear the brunt of disease burden perpetuating vicious cycle of malaria and poverty [10,11]. To address gamut of these issues; information, education, and communication (IEC) activities were identified as the integral component of integrated disease vector management approach for educating communities as demonstrative project based in Assam [12]. Included in this communication is the multi-pronged strategy that helped elicit community participation as well as building capacities of healthcare providers enabling roll back malaria in malaria-endemic block of Kamrup district of Assam, North-East India.

Health seeking behaviour

In NE India, majority of the tribal settlements are generally located in interior villages/ hamlets in juxtaposition to forest-fringe areas with poor road connectivity whereas healthcare facilities are mostly centred in town areas. Dimoria (Kamrup district) block of Assam is one such 'Integrated Tribal Development Project' area which was adversely affected by malaria reporting persistent transmission and focal disease outbreaks few years apart. A study based in this block (population ~1,00,000) revealed that while prevalence of malaria was significantly high in tribal villages, cases were few and far in town just few kilometres apart as if malaria is only a kilometre away [13]. It was observed that during focal outbreaks of households inspected, many just not approached healthcare facility rather stayed indoors for wide array of reasons: (i) misbelief that it is a curse of God, (ii) for being inconvenient to walk long distances and/or lack of public transport/motorable road, (iii) lack of resource to pay treatment costs often with fatal outcome that remained largely unreported [9,14]. Among those reporting fever, majority (773/869, 89%) opted for government facility (because it is free), 6.7% sought treatment form qualified private practitioners, 3.4% approached pharmacies (unqualified doctors), while 0.8% opted self-medication based on local remedies. What even more critical was the time gap between onset of fever and reporting that varied from one day (8.6%), 2 days (30.7%), 3 days (14.5%) and 4 days (46%). Late reporting often held due of ignorance of the disease outcome was the underlying cause of severe complications/pernicious malaria/death. Of those reached the nearest healthcare facility, only 39.4% had their blood-smear examined and the rest received symptomatic treatment. Of available treatment options, most (63%) preferred tablets over to parenteral and had spent between US $ 0.50 - 90.00 dollars per episode in relation to severity/clinical presentation. For preventive measures, most respondents (99.6%) used mosquito nets but only 64.7% of households permitted indoor residual sprays of DDT. The compliance to treatment schedule was far from satisfactory so much so that only 28% had taken the full regimen of prescribed anti-malarial drugs resulting in incomplete treatment at large. It was apparent that there was a clear need of educating communities for extended cooperation on malaria prevention and control that remained neglect for long.

Health education: Anti-malaria month

Taking cognizance of treatment seeking behaviour, integrated vector management(IVM) approach was put into practice of which health education and confidence building measures were taken head on prioritizing high-risk communities eliciting community involvement (Figure 1). Health camps were organized in coordination with state health services, non-government organizations (NGOs)/faith-based establishments for creating general health awareness on malaria and preventive measures.

In these camps, live demos were arranged on mosquito life cycle stages along with malaria parasite characteristics and preventive aspects supported by exhibits and screening of video films (Figure 2). Variety of public health education materials, viz., booklets, pamphlets, video films, TV spots, web-posts were developed in local language (Assamese), Hindi and English for larger circulation detailed as below:

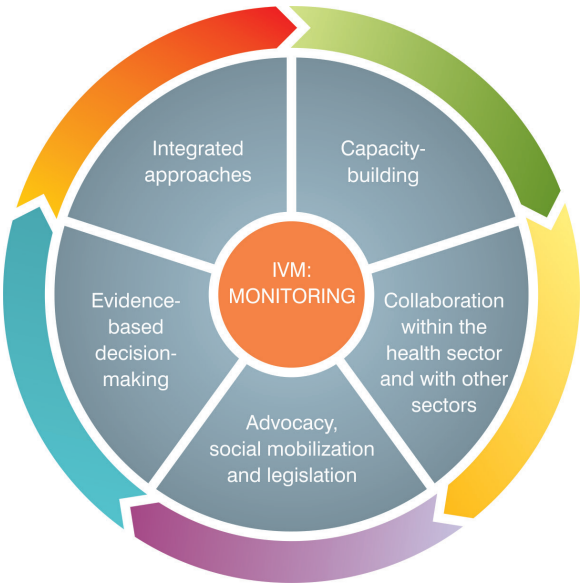


Figure 1. Components of integrated vector management for disease transmission control. Source Reference [15]

1. Booklet "Pyrethroid impregnated mosquito nets, protection from mosquitoes and malaria". Technical Information Series No. 002/96. Available at the ICMR - National Institute of Malaria Research, New Delhi.
2. Video film "Insecticide impregnated bednets for malaria control" in English, Hindi and Assamese, Master Tape No. 2006, 2008, 2061. Available at the ICMR - National Institute of Malaria Research, New Delhi.
3. Malaria Bednets, a TV spot (English, Hindi). Master tape No. 2013, 2072. Available at the ICMR - National Institute of Malaria Research, New Delhi.
4. Popular article on "Roll Back Malaria", the Assam Tribune dated 20 November 2000, Vol. 62, No. 316, page 4.
5. Pyrethroid impregnated mosquito nets for malaria control in North-eastern India. A success story by Vas Dev post at RBM website (www.rbm.who.int) during July/August 2002 under Partnership at Work.
6. Malaria Control Activities in Kamrup, state of Assam (India), IMAGE GALLERY post at RBM website (www.rbm.who.int) during February/March 2003 under Partnership at Work.

In addition, door-to-door malarial fever surveys/mass blood surveys were conducted in high-risk villages providing diagnosis and treatment gratis as confidence building measures in the ethnic communities (Figure 3). Group meetings were held in collaboration with village heads and local community leaders seeking access to communities, extended cooperation, and much needed compliance. All these health education activities were intensified during anti-malaria month observed in June each year corresponding to high transmission period in collaboration with stakeholders. During this period, news-media coverages including popular articles in local dailies, TV spots, and documentary on malaria prevention were telecasted on multiple occasions both on regional and national networks

Figure 2. Health camp in malaria-endemic village; Top: malaria clinic service for case detection and treatment; Bottom: exhibition demonstrating mosquito life cycle and malaria parasite in malaria endemic villages of Assam, North-East India.

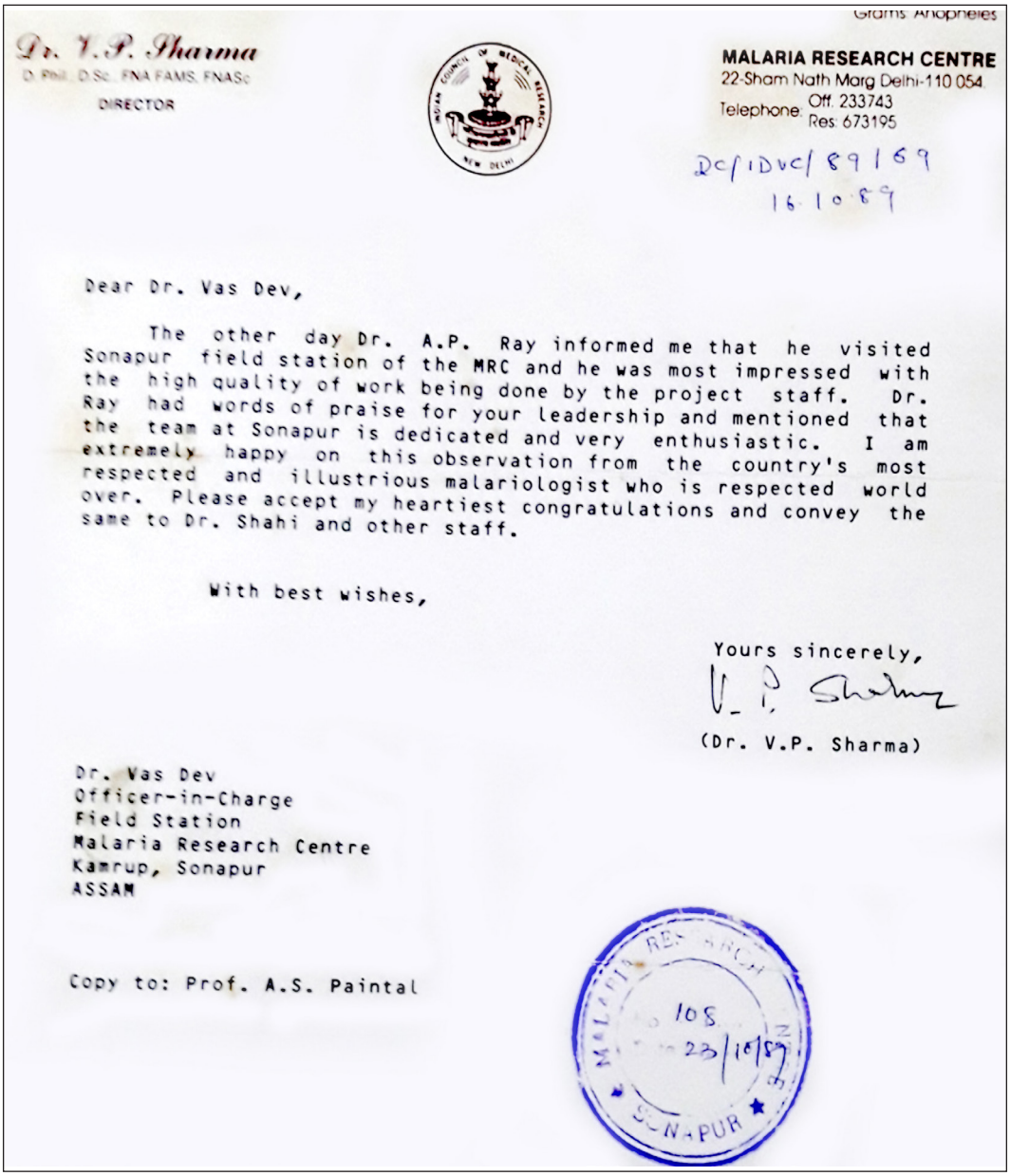
during prime time for wider publicity/dissemination of recent research findings and applications.These measures years together resulted in substantial increase in awareness evidenced by increased access to communities (formerly intractable) and sound knowledge on disease vectors and preventive aspects (Box 1). Having accomplished confidence of the communities, alternative intervention based on insecticide-treated nets (ITNs) was launched in high-risk villages as demonstrative research project in lieu of DDT residual spraying to assess impact on malaria transmission and social acceptability. The next step in order was to mobilize communities for increased participation for vector control making malaria prevention everyone's concern to promote the RBM initiative, '*zero malaria starts with me*' seeking wholesome participation.

Figure 3. Top: Mass blood surveys for malaria detection; Bottom: administering radical treatment to malaria positive cases.

Community participation

Having observed high refusal rates to DDT indoor residual spraying, field evaluation of insecticide-treated nets was launched in selected high-risk villages for which community participation was deemed necessary for successful execution. In this exercise community-owned nets were manually treated with pyrethroid every six-month for maintaining residual efficacy against the target vector mosquito, *Anopheles minimus*, an efficient vector of malaria in NE India [16].

Box 1.



Grams: Anopheles

Dr. V.P. Sharma
D. Phil., D.Sc., FNA, FAMS, FNASc
DIRECTOR

MALARIA RESEARCH CENTRE
22-Sham Nath Marg Delhi-110 054
Telephone: Off. 233743
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Dc/IDvc/89/69
16.10.89

Dear Dr. Vas Dev,

The other day Dr. A.P. Ray informed me that he visited Sonapur field station of the MRC and he was most impressed with the high quality of work being done by the project staff. Dr. Ray had words of praise for your leadership and mentioned that the team at Sonapur is dedicated and very enthusiastic. I am extremely happy on this observation from the country's most respected and illustrious malariologist who is respected world over. Please accept my heartiest congratulations and convey the same to Dr. Shahi and other staff.

With best wishes,

Yours sincerely,
V. P. Sharma
(Dr. V.P. Sharma)

Dr. Vas Dev
Officer-in-Charge
Field Station
Malaria Research Centre
Kamrup, Sonapur
ASSAM

Copy to: Prof. A.S. Paintal

MALARIA RESEARCH CENTRE
105
23/10/89
SONAPUR

Having realized the perceived benefits of relative freedom from malaria and decreased nuisance of biting insects, communities extended full cooperation in having their net impregnated periodically and much needed compliance for using these nets regularly for personal protection for self and family (Figure 4). Massive drive was launched by student/public participation in endemic villages for increased mosquito net ownership and utilization of net-based intervention to prevent mosquito bites (Figure 5). Community participation was forthcoming, and responses were overwhelming. Mass net-impregnation in endemic communities resulted in appreciable transmission reduction and general wellbeing generating additional demands for impregnated bed-net supply in risk communities [17,18].

Figure 4. Top: Community-owned nets being treated with pyrethroid manually as demonstrative exercise; Bottom: village communities engaged in having their net impregnated at health camp.

Human resource development

Human resource development and monitoring are key components of the national control programme to stay updated with the latest developments in diagnosis and treatment aspects. It is a continuing educational activity for benefit of the existing staff strength as well as new recruits for which number of sponsored activities were organized for each category of staffs annually [19]. While expertise on classical mosquito taxonomy is getting scarce

Table 1. List of workshops, seminars and training and re-orientation programme organized during 1992-2012.

S. N.	Title	Dates	Venue	No. of Participants	Sponsor
1.	One day Seminar on "Management of severe and complicated malaria" for Tea Garden doctors	May 19-20, 1992	Thakur Bari Club, Ranga Para& East Boroi Club, Biswanath Circle, Assam	53	Indian Tea Association (ABITA)
2.	Three days' Workshop on 'Integrated Disease Vector Control of Malaria' for health and Non-Health Officials.	March 10-12, 1993	Assam Administrative Staff College, Khanapara, Guwahati.	30	WHO (SEARO)
3.	One Day Seminar on 'Malaria and its containment' for Tea Garden Doctors.	May 21, 1993	Thakur Bari Club, Ranga Para, Tezpur, Assam	100	India Tea Association (ABITA)
4.	ThreeDays Workshop on 'Integrated Disease Vector Control of Malaria' for Defence personnel	September 13-15, 1993	151 Base Hospital, Vasishta, Guwahati	25	Ministry of Defence, Govt. of India
5.	Malaria Microscopy training for Lab. Technicians.	December 1–30, 1995	Malaria Research Centre, Sonapur	11	Govt. of Arunachal Pradesh
6.	Malaria Microscopy training for Health Workers of Tea Estates of Darang Circle, Assam	December 14-28, 1998	Mangal Doi Circle District Hqs.	13	Indian Tea Association (ABITA)
7.	Training on *Plasmodium falciparum* monitoring for Microscopists of Assam.	January 1-24, 1999	Malaria Research Centre, Sonapur	16	World Bank/ NAMP
8.	Training on Malaria Microscopy at Induction level for State Lab. Technicians.	January 24-5 Feb. 2000.	Malaria Research Centre, Sonapur	21	World Bank/ NAMP
9.	Training on Malaria Microscopy for State Lab. Technicians (Re-orientation).	February 7-11, 2000	Malaria Research Centre, Sonapur	20	World Bank/ NAMP
10.	Training on Malaria Microscopy for Rural Health Volunteers (induction level)	May 19–30, 2000	Akajan, Dhemaji	20	Rural Volunteer Centre (NGO)
11.	Training on Malaria microscopy of Volunteers of Gyan Vigyan Samiti	Nov. 13–16, 2000	Malaria Research Centre, Sonapur	09	Gyan Vigyan Samiti (NGO)
12.	Training on Malaria Microscopy for Technicians of Tea Garden Hospitals	Nov. 17–18, 2000	Dirial Central Hospital, Dibrugarh	19	Indian Tea Association, Assam
13.	Training on Malaria Microscopy for Technicians of Tea Garden Hospitals	May 19-30, 2002	Margherita Central Hospital	20	Indian Tea Association, Assam
14.	Refresher course on Malaria Microscopy for Volunteers of NGO based in North-east	Jan. 8–12, 2007 & April 23–27, 2007	Malaria Research Centre, Sonapur	46	World Vision (NGO)
15.	Training on Malaria Microscopy for fresh recruits of batches Technicians under NRHM, Assam	March 13 – April 3, 2008	Regional Training Centre, Health & FW, Khanapara, Assam	150	NRHM, Assam
16.	Re-orientation Training programme (5 days) for State Lab Technicians on Malaria microscopy	Nov - Dec 2008	Regional Director (H&FW), GOI, Khanapara, Guwahati, Assam	40	Assam State Vector Borne Disease Control Society
17.	Re-orientation Training programme (5 days) for State Lab Technicians on Malaria microscopy (3 batches)	March – May 2010	National Institute of Malaria Research, Guwahati	65	Assam State Vector Borne Disease Control Society
18.	Re-orientation Training programme (5 days) for State Lab Technicians on Malaria microscopy (1 batches)	March 26–30, 2012	National Institute of Malaria Research, Guwahati	19	Assam State Vector Borne Disease Control Society

on account of attrition of skilled workforce, newer innovations are evolving for reaching confirmed identification of mosquito species (molecular taxonomy) and malaria parasite diagnostics like rapid diagnostic kits (RDKs), and control aspects for vector containment (ITNs/LLINs). In this context, series of workshops, seminars, training and re-orientation programmes, refresher courses, group discussion forums were organized for benefit of state health personnel, medical college staffs, public and private sectors alike supported by lecture materials, practical classes, video films on the subject and field visits (Table 1) (Figure 6).

Figure 5. Top: Procession by school students for increased awareness on malaria prevention in malaria-endemic villages. Bottom: Mother and child and family folks alike perceived the benefits of using insecticide-treated nets as personal guard against malaria.

Intersectoral convergence

To make 'malaria control everyone's concern, active participation was sought from major sectors and establishments alike to raise awareness on the subject. These included (i) Tea Garden industry (major industry in Assam), (ii) small scale industrial units, viz., Coal, Brick, Plywood, Leather, (iii) Defence services (Army and Paramilitary battalions), (iv) N.F. Railways, (v) Oil and Natural Gas Commission (ONGC) and Oil Refineries, (vi) Hydroelectric project sites, and above all (vii) non-government organizations (NGOs).

Many of these agencies were adversely affected by malaria morbidity amounting to increased absenteeism and decreased productivity on account of sick days. Effective liaison was established with these organizations providing services for malaria containment and sharing knowledge and expertise on the subject with positive outcome evidenced by substantial transmission reduction (Box 2). Group meetings/discussions were tailored to meet the specific needs of the individual organization including onsite demos, lectures, and interactive forums.

Figure 6. Human resource development. Top: re-orientation of state laboratory technicians on malaria microscopy. Bottom: demo on malaria parasite species identification to young recruits.

In addition, number of collaborative research projects were undertaken with other research organizations including ICMR-Regional Medical Research Centre, Dibrugarh; CRPF Base Hospital, Guwahati; All India Institute of Medical Sciences, New Delhi; Gauhati Medical College Hospital; and above all State Health Directorate of all seven states of North-East region to meet the specific needs of the control programme. Galvanizing political commitment for larger share of allocation of resources and mass movement engaging multisectoral and community mobilization have become central to the dogma of 'Zero Malaria Starts with Me' for strengthening health systems and optimal outcome (Figure 7) [20].

Box 2.

ASSAM BRANCH INDIAN TEA ASSOCIATION

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RES. (SECY) 561786
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BRANCH HEAD QUARTERS GUWAHATI
ZONAL OFFICES :
ZONE 1 : DIBRUGARH
ZONE 2 : JORHAT
ZONE 3 : TEZPUR

OFFICE OF
THE BRANCH SECRETARY
A. B. I. T. A
POST BOX NO. 1
P.O. BAMUNIMAIDAM
GUWAHATI-781 02 ASSAM

No.L/E-7/988 October 23,

Dr. Vas Dev,
Officer-in-Charge,
Malaria Research Centre,
Sonapur - 782 402.
Dist. Kamrup.

Dear Sir,

MALARIA

We quote below the relevant extract from the Minutes of the meeting of the Branch Executive Committee held on 17th September,199 for your information and record.

"The Branch Chairman also recorded deep appreciation of all members for the good work done by the Malaria Research Centre at Sonapur which would always come forward to help the industry whenever there was any problem in any of the ABITA member gardens."

2. Branch also records its deep appreciation and thanks for your continued help and co-operation in combating the disease.

Yours faithfully,

(Robin Borthakur)
Secretary

Conclusions, and specific recommendations

'Health For All'; no programme can succeed without community involvement and awareness for enhanced compliance for which communities must be empowered and engaged in the decision-making process (Figure 8). It is time to think beyond box 'one-size-fits-all' rather work with communities at centre stage for targeted action and optimal

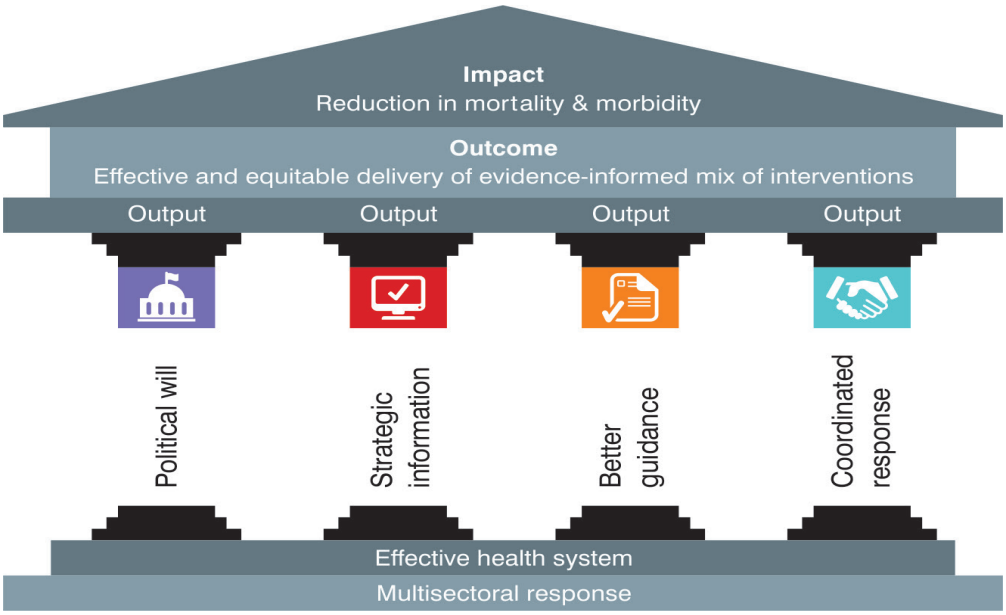

Figure 7. Multisectoral response: Source: World Malaria Report 2020. Available from:https://www.who.int/docs/default-source/malaria/world-malaria-reports/9789240015791-double-page-view.pdf?sfvrsn=2c24349d_5. Source Reference [20].

Figure 8. Critical elements of 'Health for All' model. Source: Wikipedia

outcome. Community-based malaria control in India has been resounding success in reducing malaria morbidity and linked to income generating schemes reposing confidence in risk population groups [21-23]. Malaria is preventable and curable illness, yet it continued inflicting morbidity for decades much due to lack of awareness and community participation.

No health programme can succeed in isolation [24]. There is no single magic bullet, rather it requires unified efforts and convergence of stakeholders mitigating the disease risk [25,26]. It is imperative that educating communities is cardinal to access for extended cooperation for decisive attack on disease vectors to disrupt malaria transmissionor per se any other disease control programme [27]. Equally important would be the continuing education programme for upgrading skills of health personnel at various echelons of programme operation for delivery of quality healthcare services [28,29]. Attrition of skilled health staffs looms large and must be heeded to maintain continuing services for benefit of communities at large. Disease burden is unevenly distributed with large case load in remote outreach population groups for which role of NGOs is critical ensuring services uninterrupted where disease burden is the most. Given the modern-day communication means communities today are better informed and forthcoming, yet access to treatment and prevention are far from satisfactory [30]. With the current tools and knowledge on disease vectors and parasite biology [31,32], substantial transmission reduction has been achieved at the sub-national level along with increased awareness on disease prevention and control [33]. As many 40 countries are certified malaria-free (China being the latest entrant declared malaria-free in 2021), more are inching towards the goal of malaria elimination [20]. India too is reporting declining transmission trends [34],yet there are multiple challenges to reach the last mile for which the set deadline for achieving zero transmission by 2027 is approaching fast [35,36]. Given the window of opportunity there is no room for complacency for which it is high time to step up efforts in outreach health programmes ensuring access to each one everyone aided by information, education, and communication means; together we can defeat malaria [37,38].

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17

Emerging challenges and prospects of malaria elimination in North-East India

Introduction

Control of malaria in North-East (NE) India is of paramount importance for consistently contributing proportionally larger share of reported *Plasmodium falciparum* and attributable deaths in the country and more so to arrest the development and spread of drug-resistant malaria [1,2]. NE region (a group of seven sister states namely Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland and Tripura) is of economic importance for vast forest cover and abundant natural resources (oil & natural gas, coal and timber), tea industry (major cash crop) and strategic significance sharing international border (90% of its boundaries) with other member states of the World Health Organization (WHO) South-East Asia Region (SEAR). Disease transmission is perennial in the valleys, yet the distribution of cases is heterogenous with a large concentration of cases and heightened risk in forest-fringe cross-border population groups reporting focal outbreaks [3]. However, with the rollout of present-day intervention tools aided by the Global Fund to fight Aids, Tuberculosis and Malaria (GFATM) including long-lasting insecticidal nets (LLINs) for vector control and artemisinin-based combination therapy (ACT) for treatment of cases, impressive gains have been made in reducing transmission, yet there are multiple challenges on road to elimination calling for concerted action to defeat malaria [4]. Enumerated below are some of the emerging issues and priorities for benefit of the control programme to help formulate informed policies and strengthening healthcare services for universal access paving the way to end malaria.

Current status: epidemiological data appraisal

North-East India (22^0N – $29^05'$N latitude and 88^0 E - $97^03^0'$E longitude) is co-endemic for both *P. falciparum* and *P. vivax*, distribution of parasite species and transmission intensities, however, varied across states [5]. Of these, *P. falciparum* was the predominant infection in Meghalaya, Mizoram and Tripura (90%), all sharing border with Bangladesh; conversely, *P. vivax* was abundant in sub-tropical climatic zones in states of Arunachal Pradesh and Nagaland. But, Manipur reports both parasite species occurred nearly in equal proportions, while in Assam *P. vivax* proportions remained $\leq$30%[6]. In 2020, collectively all seven sister states including Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram and Tripura that makeup NE (nearly 4% of the country's population) contributed 7.4% of cases of which 85% were due to *P. falciparum* alone (Table 1). Malaria-attributable deaths were solely ascribed to *P. falciparum* associated with disease severity and associated complications.

Table 1. Relative contribution of malaria cases and attributable mortality from North-Eastern states of India for data based on 2020*

No. of cases	India (pop. ~1.37 billion)	North-Eastern states (pop. ~52 million)	% Share contribution of cases from North-Eastern states (pop. ~4 %)
Total malaria	186,532	13,759	7.38
P. falciparum	119,088	11,662	9.79
No. of total deaths	93	16	17.20

*Source: National Vector Borne Disease Control Programme, India. Source Reference [2]

All states reported cases associated with persistent transmission of the causative parasites with seasonal peak (April – September), yet Meghalaya, Mizoram and Tripura contributed the bulk of disease burden, mostly *P. falciparum* (Table 2). Nevertheless, given the rollout of LLINs and ACT, disease transmission trends were clearing deaccelerating post 2016 (Figure 1). Among NE states, Assam which routinely contributed 50% of the total cases from the NE region, has achieved substantial transmission reduction since 2006. The number of cases declined by >90% in 2020 compared to the 2016 level which was once considered intractable with a history of focal disease outbreaks (Figure 2). Most districts are currently reporting <1 annual parasite incidence (API) per thousand population. The persistence transmission of *P. vivax*, however, would emerge the challenging task until anti-relapse treatments are made available that are safe and effective.

Among others, there are several confronting issues detailed as below which should be attended helping make informed decisions to end transmission in the given time frame by 2030.

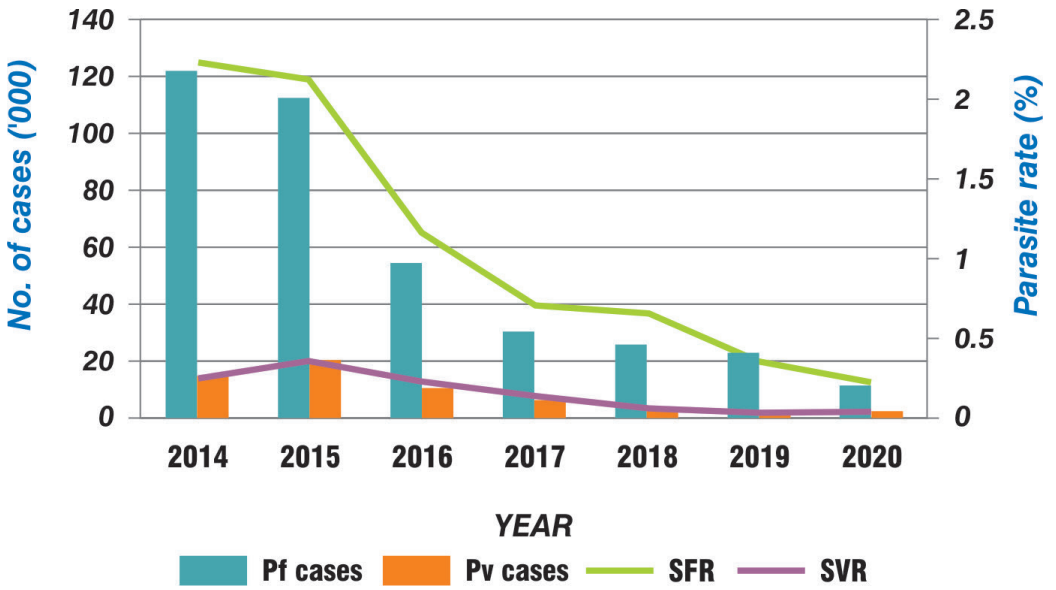

Figure 1. Reducing trends of malaria transmission in North-Eastern states based on cumulative data of the seven sister states, including Arunachal Pradesh, Assam, Meghalaya, Mizoram, Nagaland, Manipur and Tripura (2014-2020). SFR (smear falciparum rate) and SVR (smear vivax rate) denote % blood-smears positive for malaria parasite. Pf = *Plasmodium falciparum*, Pv = *Plasmodium vivax*. Data source: National Vector Borne Disease Control Programme, India. Source Reference [2]

Table 2. Comparative epidemiological data of malaria transmission in North-Eastern states of India during (2016-2020)*

State	Year	Pop. (millions)	No. of blood-smears examined for malaria parasite	No. of malaria positive cases		Annual blood-smear examination rate (%)**	Annual parasite incidence***	No. of malaria-attributable deaths
				P. falciparum	*P. vivax*			
Arunachal Pradesh	2016	1.57	151,590	895	2,233	9.65	1.99	2
	2017	1.60	153,620	488	1,058	9.60	0.97	0
	2018	1.63	145,353	154	471	8.91	0.38	0
	2019	1.67	139,689	27	112	8.36	0.08	0
	2020	1.71	53,069	7	24	3.10	0.02	0
Assam	2016	33.90	3,032,997	5,686	2,140	8.94	0.23	6
	2017	34.49	2,669,423	3,494	1,787	7.74	0.15	0
	2018	35.01	2,364,621	2,859	957	6.75	0.11	2
	2019	35.60	4,352,477	872	587	12.22	0.04	4
	2020	36.02	3,246,745	266	218	9.01	0.01	2
Manipur	2016	2.97	94,115	58	64	3.17	0.04	0
	2017	3.21	115,733	22	58	3.60	0.02	0
	2018	3.27	98,375	3	9	3.01	0.004	0
	2019	3.37	231,833	5	11	6.88	0.004	0
	2020	3.50	166,260	13	10	4.75	0.006	2
Meghalaya	2016	3.21	468,254	31,867	3,280	14.58	10.95	45
	2017	3.47	421,145	14,418	2,036	12.14	4.74	12
	2018	3.53	326,051	6,065	329	9.23	1.81	6
	2019	3.68	422,237	2,364	251	11.47	0.71	4
	2020	3.77	327,132	1638	245	8.68	0.50	4
Mizoram	2016	1.20	267,747	5,907	1,676	22.31	6.32	9
	2017	1.23	213,601	4,974	741	17.37	4.65	4
	2018	1.26	218,178	3,937	359	17.31	3.41	3
	2019	1.28	232,916	8,010	533	18.19	6.67	8
	2020	1.30	277,834	6811	1538	21.37	6.42	1
Nagaland	2016	2.98	252,232	316	512	8.46	0.28	0
	2017	3.12	254,038	188	206	8.14	0.13	1
	2018	3.20	255,888	24	89	7.99	0.03	0
	2019	3.27	225,519	4	16	6.89	0.006	0
	2020	3.34	67,559	9	3	2.02	0.004	0
Tripura	2016	3.94	351,392	9,545	1,001	8.91	2.68	14
	2017	4.42	392,452	6,571	480	8.87	1.59	6
	2018	4.54	483,982	12,600	479	10.66	2.88	13
	2019	4.68	619,912	11,636	801	13.24	2.66	1
	2020	4.79	415,379	3,100	295	8.67	0.71	2

*Source: National Vector Borne Disease Control Programme, India, **% of population screened for malaria parasite, ***number of malaria cases per thousand population. Source Reference [2]

Drug-resistant malaria and treatment policy

NE region is considered a corridor for the spread of drug-resistant malaria to peninsular India emanating from adjoining SEAR countries. *P. falciparum* is the predominant infection

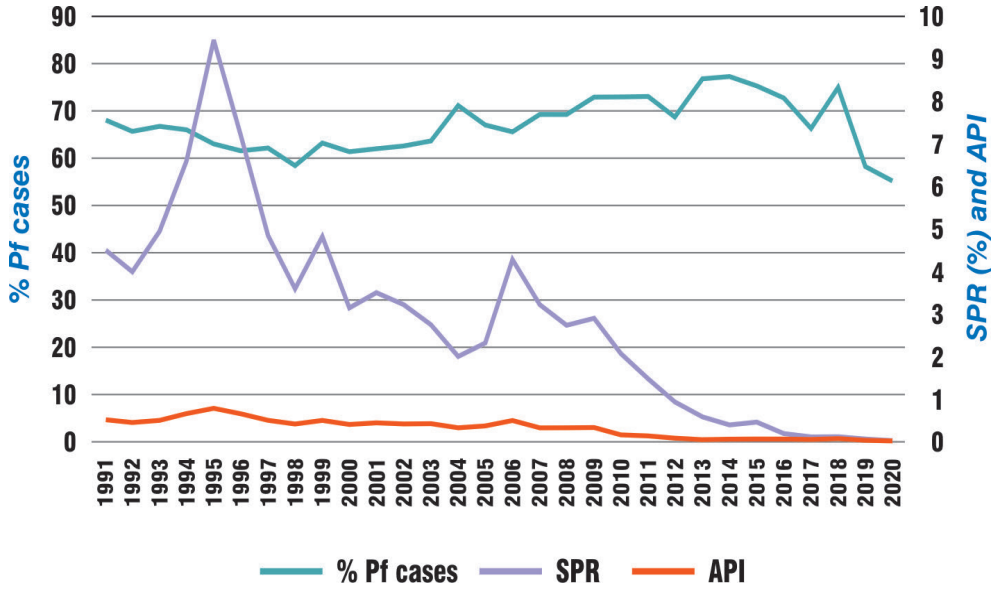

Figure 2. Malaria transmission in Assam (1991-2020). SPR denotes smear positivity rate of blood-smears examined for malaria parasite. Pf = *Plasmodium falciparum*. % Pf relates to per cent of *P. falciparum* cases of total positive cases for any malaria parasite. API denotes number of malaria positive cases per 1,000 population/year. Data source: State Vector Borne Disease Control Programme of Assam. Source Reference [2]

associated with disease severity and extended morbidity. Given the advent of ACTs, the disease transmission trends are declining, but *P. falciparum* remained the predominant infection proportions of which were seen increasing unabated (Figure 3). Drug-resistance malaria (invariably refers to *P. falciparum*) was first detected in Assam in 1973, has evolved considerably from mono-to-multidrug resistant strains over time [7,8]. Focal outbreaks were largely ascribed to an unprecedented rise in *P. falciparum* cases evidenced by epidemiological data on disease transmission and attributable mortality [9]. Periodic monitoring of therapeutic efficacy of antimalarials revealed that parasite is continually evolving mandating upgradation of drug-policy ensuring radical cure before these become widespread [10]. The problem is acute closer to the international borders marked by high transmission intensities due to lack of adequate infrastructure and access to treatment and prevention [11]. The population groups in these areas are highly receptive to malaria due to lack of awareness and remain devoid of treatment months together amounting to build-up of parasite reservoir and persistent transmission [12,13]. There are already reports of emerging artemisinin resistance evidenced by molecular markers specific to the NE region [14]. Arresting the development and spread of drug-resistance is cardinal to malaria elimination initiative for which monitoring and appropriate drug-policy in place are tantamount to disrupt transmission and should be the continuing activity of the control programme [15].

Targeting asymptomatic malaria

Asymptomatic malaria is much more abundant than believed, leave alone the identification and delimitation of such population groups for targeting treatment, there are no plans

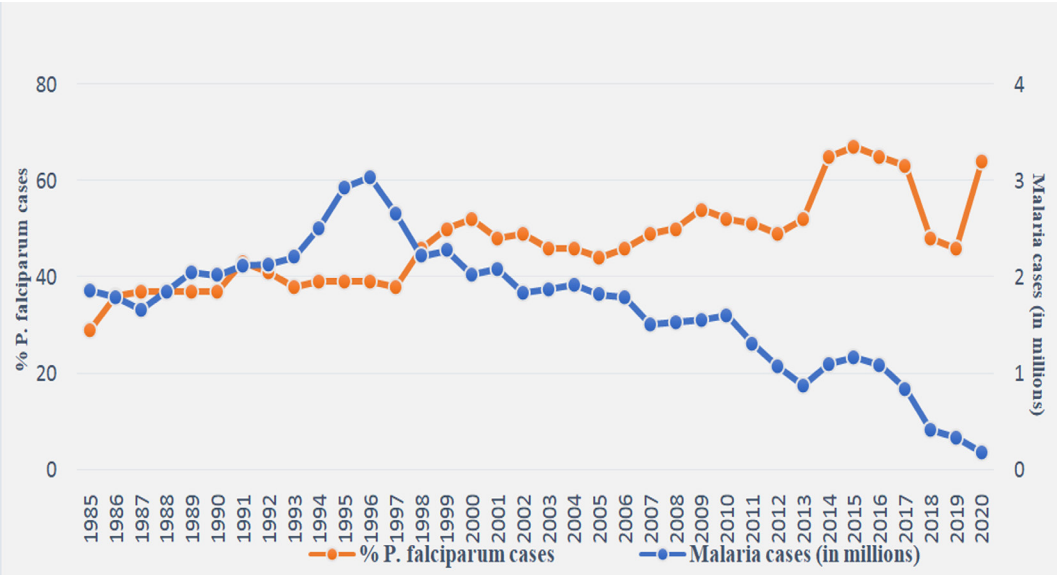

Figure 3. Rising proportions of *Plasmodium falciparum* cases in India. Data source: National Vector Borne Disease Control Programme. While malaria cases have consistently declined from 1996 onwards, *P. falciparum* prevalence have steadily increased from 29% in 1985 to 64% in 2020. Source Reference [2]

on the anvil to eliminate such foci [16]. Populations living in territories experiencing high transmission intensities and suboptimal interventions for decades have turned asymptomatic carriers – a source of parasite reservoir for persistent transmission [17]. Due to low literacy levels and lack of awareness on disease prevention and control, these groups are less likely to seek treatment. Furthermore, healthcare facilities are too often distant and grossly inadequate which are usually centred in town areas. As such disease surveillance is far from complete leaving many groups unattended due to operational constraints, viz., lack of skilled human resources, poor access to villages due to social conflicts and inadequate logistic supplies etc. As much as 30% of population is estimated to be asymptomatic in certain high-risk districts for which there exists no surveillance mechanism to test, treat and track. Disease burden is enormous due to the hidden sea of parasites and remains poorly addressed. There is an apparent need to intensify disease surveillance and meet logistics to liquidate these foci enabling end transmission [18].

Ecological succession of disease vectors

Anopheles minimus and *An. baimaii* are proven vectors of malaria evidenced by records of seasonal abundance and incrimination across NE states [19]. Of late, the NE region is experiencing an economic boom associated with infrastructure expansion and population growth resulting in ecological changes at expense of deforestation and human migration/ increased agricultural acreage. Both *An. minimus* and *An. baimaii* are efficient vectors and highly susceptible to DDT as well as other residual insecticides including pyrethroids, scaling up of which has resulted in the diminution of populations below the threshold density. Instead, *An. culicifacies* (the predominant vector in mainland India) has invaded

degraded forests of NE and establishing evidenced by building population densities and seasonal infections [20-22]. *An. culicifacies* is ascertained to be multi-resistant to available arsenal of insecticides and held less amenable to control interventions [23]. There is every possibility of invasion by *An. stephensi* (the urban vector in India), hitherto unreported in the NE region, due to continued urbanization and increased travel and trade [24]. Faunistic changes are happening inadvertently having implication in disease transmission and control strategies [25,26]. What would be critical is to maintain vigil by monitoring population dynamics and susceptibility status of vector populations helping institute appropriate interventions in time and place averting impending disease outbreaks and spread of malaria [27].

Strengthening cross-border collaboration

NE shares a wide international border with Bhutan to the west, Myanmar to the east and Bangladesh to the south, and have documented history of transmission of drug-resistant malaria for which this region is considered corridor of spread to peninsular India and beyond. Borders are porous and human exodus is imperative given the compelling socio-economic conditions for subsistence in adjoining countries. Mosquitos' sans borders: disease vectors and ecology are very similar on both sides helping facilitate entry of varied parasite strains mixing of which results in explosive disease outbreaks. There are records of fulminating outbreaks and occurrence of drug-resistance of high magnitude resulting in heightened morbidity in bordering population groups [28]. Cross-border areas are often devoid of adequate healthcare facilities to meet the contingencies in complex emergencies. These areas are assessed to be high-risk for contracting infection associated with varied risk factors, viz., location of settlements adjacent to forest-fringe and/or near to vector breeding habitat (<1 km, the mosquito flight range), distance to nearest healthcare facility >5 kms, lack of motorable roads, illiteracy; interventions in which should be prioritized to mitigate the disease onslaught preventing propagation of resistant strains [29]. Co-ordinated vector control interventions and data sharing on the therapeutic efficacy of antimalarials across borders for having uniform drug-policy as well as behavioural change communication campaigns are deemed necessary coupled with strengthened health systems to meet the logistic needs [30].

Building human resources and capacities

Building skilled human resources is an asset to the control programme for quality services for accurate diagnosis and decisive attack on disease vectors [31]. While expertise on classical taxonomy is getting scarce, skilled work force at various levels of programme operation is waning due to attrition. The newer interventional technologies are emerging for which upgrading skills of healthcare personnel is vital to the programme and should be the continuing activity. Accurate diagnosis of malaria parasite would emerge as a challenge as parasite become scarce on account of elimination initiative. Disease surveillance should be the core-activity and not compromised rather it should be strengthened reaching out the outreach marginalized population groups. Entomological surveillance is virtually non-

existent what should have been the core-activity at the zonal level [23]. Targeting species-specific interventions are cost-savvy and should be prioritized averting impending disease outbreaks. Training and re-orientation programmes should be extended to supporting staffs/agencies, viz., accredited social health activists (ASHA), non-government organisations (NGOs), village link workers (VLWs), volunteers, community leaders alike for creating enabling environment for greater reach of the programme seeking community participation and much needed community compliance. Intersectoral convergence involving multiple organizations, stakeholders, viz., defence establishments, small-scale industrial units, public and private sector alike should be promoted helping make informed decisions to prevent malaria.

Conclusions, and the way forward

NE India has registered a notable reduction in disease transmission barring few inter-border districts of Mizoram, Tripura and Meghalaya where the risk of malaria is still high [1,2]. Assam has made huge strides in reducing transmission (the most populous state constitution 65% population share of NE region) and fast approaching pre-elimination stage at the sub-national level. Complacency at this stage would cost dearly rather it is the time to seize the opportunity for upscaling interventions towards universal coverage. Unstinted political commitment is need of the hour for greater allocation of resources ensuring logistic supplies uninterrupted to keep malaria at bay. There is a dearth of intermediate supervision (at present non-existent) and monitoring for judicious application

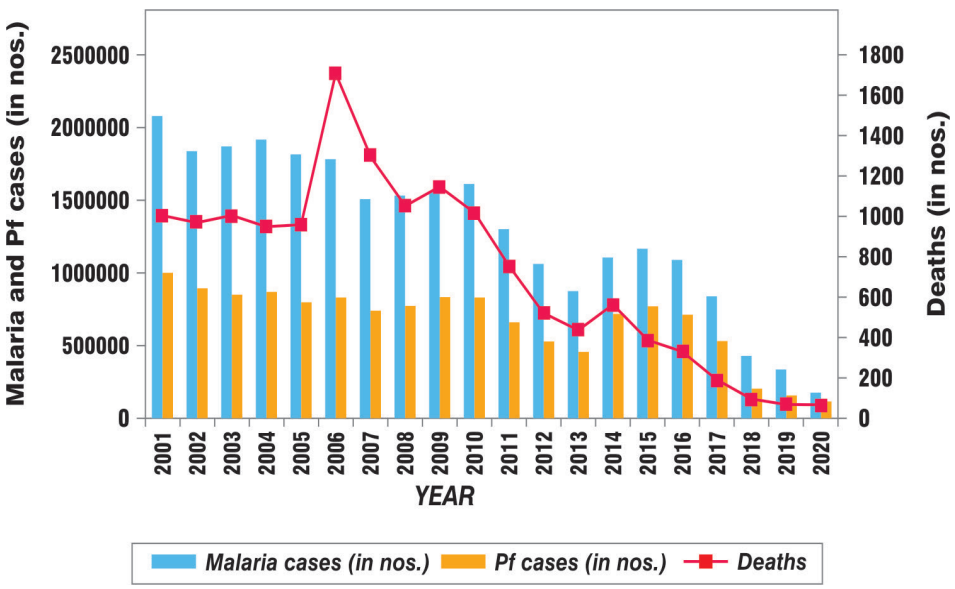

Figure 4. Declining transmission of malaria in India (2001-2020). Cases have consistently declined from 2.08 million to 0.33 million during 2001 to 2019. *Plasmodium falciparum* (Pf) cases have also declined from 1.0 to 0.15 million cases in the corresponding period. Deaths less than 2000 were reported during all these years with a peak in 2006 due to epidemic in north-eastern states (Source:https://nvbdcp.gov.in/index4.php?lang=1&level=0&linkid=420&lid=3699).

of resources in the programme execution [32]. India has made impressive gains in reducing malaria burden and stands out to be the only member among 11 'high burden to high impact (HBHI)'countries reporting a steady decline in cases and deaths corroborated by malaria-metric indicators (Figure 4,5) [33]. It is the only high endemic country which has reported a decline of 17.6% in 2019 compared to 2018. The Annual Parasitic Incidence (API) reduced by 27.6% in 2018 compared to 2017 and by 18.4% in 2019 as compared to 2018 and has sustained API <1 since 2012. In 2020, India registered >50% decline in cases compared to 2019 level [34]. During all these years, the NE region contributed significantly towards appreciable transmission reduction commencing 2006 [2].

Currently, most Indian states are reporting less than 1000 cases except a few districts with high forest cover in east-central (Chhattisgarh, Odisha, Jharkhand, West Bengal) and NE states in which just about 6.6% of the population (dominated by tribal aborigines) living in forested areas contributed 21% of cases and 53% of deaths reported in 2019 requiring focussed interventions (Figure 6) [35]. Resource investing in these states/high-risk districts would yield good dividend in reducing disease burden and containing the spread of drug-resistant malaria. In the SEAR of WHO, while other member countries approaching malaria elimination [36], India still contributes the majority of cases (80% of disease burden); true incidence, however, is estimated to be manifold than reported. WHO estimates 5.5 million cases compared to the 338,500 reported in 2019, and mortality to be 100-fold higher than 7,700 the least [33]. Nevertheless, despite the COVID-19 challenge engaging health systems overwhelmingly [37], India stands optimistic targeting elimination by 2027 with added knowledge on disease vectors and transmission dynamics [38].

Amidst HBHI countries' response, India has taken bold initiatives strengthening human resource under National Health Mission (NHM) and the Integrated Disease Surveillance Project (IDSP) for intensified disease surveillance and monitoring helping early warning

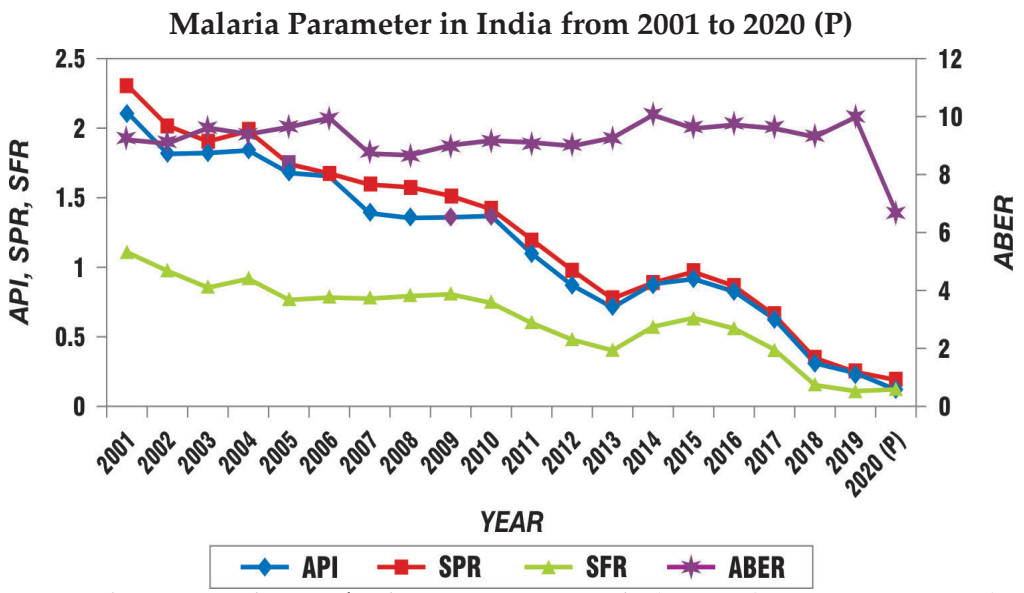

Figure 5. Malaria-metric indicators of malaria transmission in India (2001-2020). Smear positivity rate (SPR) has declined from 2.31 to 0.25, and Smear falciparum rate (SFR) has declined from 1.11 in 2001 to 0.12 in 2019. API=Annual Parasite Incidence | SPR=Smear Positivity Rate | SFR= Smear Falciparum Rate | ABER=Annual Blood-smear Examination Rate. P=Provisional data (Source:https://nvbdcp.gov.in/index4.php?lang=1&level=0&linkid=427&lid=3705).

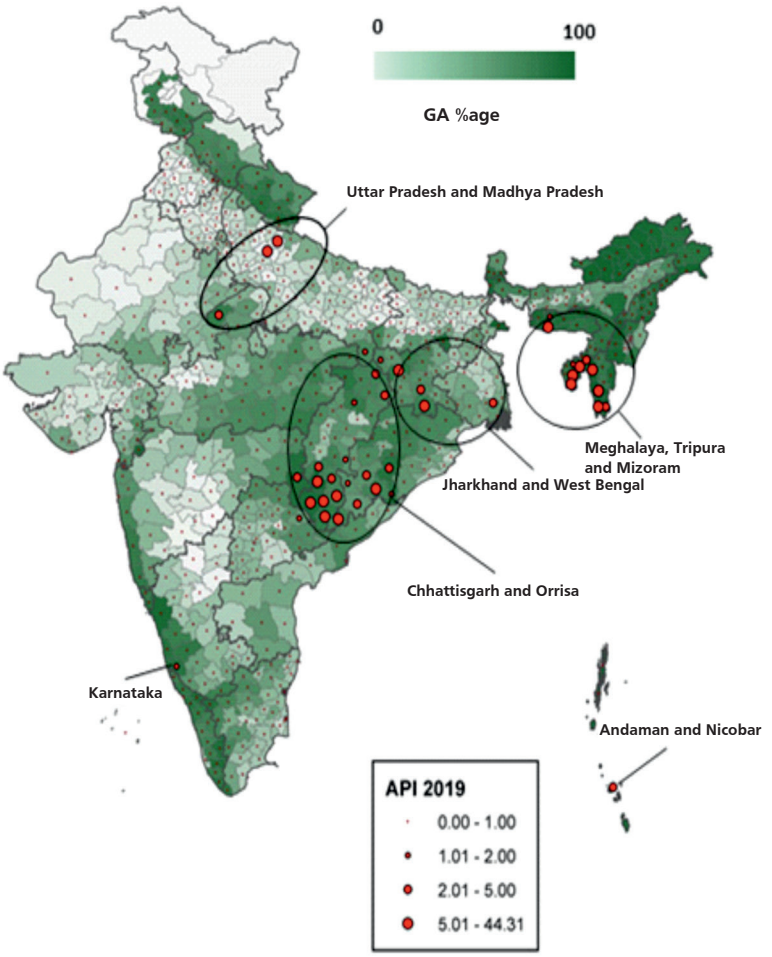

Figure 6. Percentage of geographical area (GA) with forest cover and malaria endemicity in India. Big red dots lying in the greener areas indicate high malaria endemicity in areas with very dense forest canopy. States with high malaria endemic districts are encircled. API denotes Annual Parasite Incidence per thousand population. Source reference [35].

Figure 7. Testing for malaria intensified aiming elimination by 2027 (https://www.financialexpress.com/lifestyle/health/government-launches-national-strategic-plan-for-elimination-of-malaria-plans-to-eradicate-vector-borne-disease-by-2027/761063/India).

for keeping guard of emerging threats (Figure 7). Focal disease outbreaks (what used to be news item prior to 2006) are now unheard off and have become thing of the past. Communities are better informed and healthier; participation is forthcoming and have new levels of confidence.

Not to be euphoric; certainly, newer tools and integrated approach seeking multisectoral convergence would be needed to mitigate emerging paradigms of outdoor transmission, urban malaria, multi-drug resistant malaria and insecticide resistance in disease vectors in the context of changing landscape [39,40]. Additional surveillance tools [41,42], and delivery mechanisms would be required to reach the marginalized population groups ensuing equity in healthcare access preventing malaria [43,44]. Funding gap is too far wide whereas population coverage is miniscule of what is mandated to meet healthcare needs of population at risk of malaria. There is a window of opportunity scaling up interventions in high-risk districts/population groups ensuring early case detection and prompt treatment (EDPT) targeting the asymptomatic parasite reservoirs and making treatments affordable to each one and everyone breaking the chain of malaria and poverty [45,46]. India is a partner member state to the Asia Pacific Malaria Elimination Network (APMEN) and the Asia Pacific Leaders Malaria Alliance (APLMA) for coordinated action towards a common goal of 'freedom from malaria' based on shared data and experiences helping strengthen the cross-border initiative.What concerns most is the relentless march of *P. falciparum* increasing proportions which are perceived threat to the elimination initiative for which monitoring therapeutic efficacy and staying step ahead of drug resistance should be the cornerstone to zero in malaria [47]. The deadline of malaria elimination is around the corner [48], yet miles to go to the finish line; together we can make it lest we miss the opportunity. Eliminating malaria in India would be of added advantage to neighbouring countries not only achieving maintain malaria-free status but also forbidding re-establishment of malaria transmission, a critical component in maintaining malaria-free status [49,50].

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The Way Forward

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Specific recommendations: the toolkit for malaria elimination initiative

The world malaria map is shrinking with as many as 40 endemic countries certified to be malaria-free (the latest entrant being China), and many more are on a path to elimination reporting fewer than 100 indigenous cases. India has also made laudable gains over the past few years, reporting a steady decline in cases from 0.84 million in 2017 to 0.18 million in 2020 (77% decrease) and attributable deaths from 194 to 93 (50% decrease) in the corresponding period. India is a vast country with almost a billion population at risk of malaria encompassing varied agroclimatic zones, disease vectors and transmission intensities. With the rollout of present-day intervention tools (long-lasting insecticidal nets and artemisinin-based combination therapies in particular) and available knowledge on disease vectors and transmission dynamics, many endemic states and territories are approaching near elimination. Among these, North-East (NE) zone (formerly hyperendemic for malaria) has contributed significantly to reducing case incidences aided by the Global Fund to fight Aids, Tuberculosis and Malaria (GFATM) helping upscaling interventions, yet there are a host of challenges which can be overcome by strengthening technical components of an evidence-based toolkit to defeat malaria lest we become complacent. The malaria elimination toolkit (Source: Institute for Global Health Sciences, University of California, San Francisco) focuses on three primary areas: situation assessment, tailored responses, and program management and sustainability – with the goal of building capacity and optimizing a country or district's ability to advance toward elimination. These tools have helped many malaria-endemic countries accelerate towards malaria elimination by implementation at national, provincial and district levels for which the Malaria Elimination Initiative (MEI) of Institute for Global Health Sciences, University of California, San Francisco(UCSF) offers technical assistance to support the adaption, tailoring and implementation frameworks and guides for a successful outcome. The toolkit comprises five major areas of reinforcement: (i) disease surveillance and response preparedness, (ii) monitoring drug-resistance and strengthening diagnostics, (iii) vector surveillance and control, (iv) strengthening health systems for well managed programme for improved accountability and delivery of services and, (v) advocacy, minding the financial gap and sustainability; all of which would help making informed decisions for tailoring situation-specific strategies. In NE India, while some districts (bordering Bangladesh in particular) are still hyperendemic, many districts are approaching elimination for which implementation of this toolkit would go a long way in addressing the gaps to end transmission in the region. Given below are some of the measures (adapted from MEI of UCSF) which can be integrated into the health services for benefit of control programme specific to the NE region helping attain malaria-free status.

Disease surveillance: targeted surveillance and response in high-risk populations

Disease distribution and transmission intensities are heterogenous across Indian states and surveillance are grossly inadequate often missing the high-risk populations (HRP)/mobile population groups due to accessibility and health seeking behaviour for not presenting at the nearest healthcare facility.The problem is much more acute in the NE region for having a huge population living in forest-fringe/cross-border areas with little access to healthcare services. Asymptomatic malaria is widespread and remains unattended for which there exists no surveillance. In the context of malaria elimination initiative, it is of utmost importance to target these HRPs and augment logistic supplies helping meet complex emergencies averting possible focal outbreaks. It would entail building epidemiological evidence on transmission intensities and risk factors helping determine specific needs and delivery of services mitigating the disease risk. To move forward from malaria control to malaria elimination, district level preparedness need to be strengthened based on local disease surveillance data for readiness to mount prompt response addressing financial, technical, and operational programme needs and gaps. In low-transmission districts (as there are many in NE), it would be judicious to practice Reactive Case Detection (RACD) to diagnose and treat cases as early as possible *(RACD is a form of active case detection for identifying and treating additional malaria infections from an index case in areas of low-malaria transmission. It involves investigation of passively-detected cases of malaria, e.g., patients seeking care at their local clinic to determine the suspected origin of infection and the potential risk for onward transmission of malaria. If areas are receptive, additional testing of contacts is carried out to identify and treat which reduces the severity and duration of illness and prevent the spread of infection).* Conversely, in high-risk transmission areas, interventions should be intensified and directed to dilute the infectious reservoir by opting for suitable strategies specific to the local contexts and available resources helping advance towards elimination, e.g., mass drug administration (MDA), screen and treat (SAT), seasonal malaria chemoprevention (SMC). Chemoprevention strategies are advantageous to: (i) reduce malaria transmission, (ii) reduce malaria morbidity, (iii) improve surveillance, (iv) respond to emergencies including malaria outbreaks or situations where the health system is strained (e.g., COVID-19 pandemic).

Monitoring drug-resistance and improved diagnostics

The relentless march of *Plasmodium falciparum* associated with the emergence of drug-resistant malaria is unstoppable and seen emerging threat to elimination efforts. Decrease in case incidences in the recent past is certainly good news but increasing proportions of *P. falciparum* associated with disease severity and mortality is unacceptable mandating implementation of 'test-treat-track' initiative to reach out anyone at risk of malaria. *P. falciparum* has grown mono-to-multiresistant to the available armamentarium of antimalarials and there are already signs of evolving artemisinin resistance (the widely used antimalarial globally) along NE borders (the corridor of spread to peninsular India). The inter-country borders are porous and transmission intensities are intense culminating in focal outbreaks perpetuating vicious cycles of poverty and malaria. Even though India

has registered impressive gains in reducing transmission, but caseload of 0.18 million with majority *P. falciparum* cases (64%) in 2020 is still too high to dream malaria elimination by the target date of 2027. Even though, the advent of artemisinin-based combination therapies has done the magic trick in diluting the parasite reservoir/circulating parasitaemia, yet it would be prudent to stay a step ahead of fast evolving drug-resistant malaria, which can only be achieved by intensified disease surveillance, strengthening diagnostics to characterize sub-patent parasitaemia and implementing appropriate drug-policy for radical cure.

Entomological surveillance: the guiding principle

A great deal of information has been generated on mosquito vectors, bionomics and disease transmission relationships specific to India, yet what has been lacking is the entomological surveillance albeit what should have been the guiding principle for vector control. The outcome is the inapt applications of interventions in time and place resulting in the development of insecticide resistance and continuing transmission in large tracts of land. Entomological information on disease vectors, relative density, infectivity, susceptibility status to residual insecticides, resting and breeding preferences, is crucial to the control programme not only to maximize the impact of vector control but also to ascertain impact assessment of intervention tools. Ecological succession of disease vectors replacing insecticide susceptible populations with multi-resistant is threatening the control programme. The paradigm shift of mosquito vectors from indoor resting to outdoors has opened new vistas for vector control mandating newer tools to overcome residual transmission helping target deployment of appropriate interventions in time and place. Such data are deemed necessary at district/zonal level in the context of malaria elimination initiative to supplement vector control interventions to address gaps in community-wide protection.This information in combination with disease surveillance data will help in the decision making process for choice interventions to reduce human-vector contact enabling drive down transmission. Building entomological capacities and human resource development are central for data generation and assessment helping make judicious application of interventions given the limited resource envelops.

Strengthening health systems for programme reach

Distribution of malaria cases and transmission is uneven in NE states; while some districts are consistently contributing the bulk of cases whereas others are assessed to be low-risk. Cross-border populations are most at risk of malaria infection requiring prioritization for strengthening interventions and coordinated action. Nevertheless, it is essential to make a periodic situational assessment at the district level for preparedness to meet the logistic supplies, human resources for execution and supervision at various levels of programme operation. The strength of the programme lies in building capacities integrating entomological and epidemiological data at the local level translating into action by identification of risk populations and addressing financial, technical, and operational needs to sustain elimination activities. For the operational success of the programme, it is

crucial to maintain and upgrade human resource strength at state, district, health facilities down to community health workers (CHWs) for decisive action both against parasite and vector to disrupt transmission. It is the teamwork and quality of service delivery and outreach that would make a sea of difference in reaching informed decisions to improve operations and taking judicious action at the ground level. While,we can outwit malaria by joint action and commitment for leaving no one behind irrespective of the finanacial and legal stuats; lack of commitment and failed supervision at any level of operation would be detrimental to the programme.

Advocacy, the financial gap, and sustainability

Community acceptance and participation in the control programme are cardinal for successful implementation of newer tools/interventions. Community leaders/stakeholders should be part of the decision-making process which in turn would help making programs more sustainable aligned with the local context facilitating ownership. What is even more critical is to ensure 'universal coverage' reaching out to the high-risk/outreach population groups ensuring access to treatment and prevention. Coverage of at-risk populations often fell short of target owing to insufficient domestic funding and/or unsustainable donor support. The funding gap is too far wide for which suitable strategies should be in place helping transition from donors to domestic/local level funding mechanisms. It is opportune time to supplement resources apart from domestic funding and donors to meet the logistics for which intersectional convergence can help minding the funding gap and sharing programme ownership. However, translating political will into tangible action and investment would require strategic advocacy by national malaria programs and their subnational counterparts to influence governmental decision-makers and budget processes. Malaria programs may be required to strengthen subnational leadership and public financial management to ensure a resilient and sustainable malaria response at all levels. Strengthening engagement of local leaders and budgetary authorities can catalyse substantial domestic financing impact and help oversee expenditures and monitoring budget allocations. It will be necessary to engage a broader set of stakeholders for many steps along the way for no one actor can achieve advocacy goals in isolation. These stakeholders could include technical malaria partners, donor agencies, collaborating departments within the Ministry of Health, Ministry of Finance, local non-governmental and civic society organizations, local elected officials and government administrators, cross-border health counterparts, private sector companies, and community leaders.

In summary, NE has come a long way in defeating malaria post-independence (formerly considered intractable) by a tangible reduction in morbidity and mortality. It was not an easy walkway rather an arduous journey across inhospitable terrain after decades of consistent research efforts and systematic approach for field-evaluation of newer interventions that were community-based to drive down the transmission to the lowest ebb. Research and development providing evidence-based technologies hold the key in keeping malaria at bay. Building stronger health systems towards 'One Health' based on collaborative, multisectoral, and transdisciplinary approach with the goal of achieving optimal health outcomes has become central for inclusive growth enabling understanding of environmental factors that impact disease transmission.

One Health Triad: Source - Wikipedia

It is strongly advocated to invest heavily in NE India ensuring universal coverage of interventions both against parasite and disease vectors not only to disrupt transmission but also preventing re-establishment in malaria-free territories. Eliminating malaria in NE states would certainly be a distinctive achievement and could prove to be the harbinger of success to end malaria in the country.

Annexes

Annex – 1: Countries and territories certified malaria-free by WHO

Certification of malaria elimination (1955–2021):

Countries that have achieved at least 3 consecutive years of zero indigenous cases are eligible to apply for a WHO certification of malaria-free status. As many 40 malaria-endemic countries have been certified malaria-free between 1955-2021.

Country/territory	Countries certified malaria-free	Countries where malaria never existed or disappeared without specific measures
AFRICA		
Algeria	2019	
Lesotho		2012
Mauritius	1973	
La Réunion (France)	1979	
Seychelles		2012
EASTERN MEDITERRANEAN		
Bahrain		2012
Jordan		2012
Kuwait		1963
Lebanon		2012
Libya		2012
Morocco	2010	
Qatar		2012
Tunisia		2012
United Arab Emirates	2007	
EUROPE		
Albania		2012
Andorra		2012
Armenia	2011	
Austria		1963
Belarus		2012
Belgium		1963
Bosnia and Herzegovina	1973	
Bulgaria	1965	
Croatia	1973	
Cyprus	1967	
Czechia		1963
Denmark		1963
Estonia		2012
Finland		1963
France (Metropolitan)		2012

Germany		1964
Greece		2012
Hungary	1964	
Iceland		1963
Ireland		1963
Israel		2012
Italy	1970	
Kazakhstan		2012
Kyrgyzstan	2016	
Latvia		2012
Lithuania		2012
Luxembourg		2012
Malta		1963
Monaco		1963
Montenegro	1973	
Netherlands	1970	
Norway		1963
Poland	1967	
Portugal	1973	
Republic of Moldova		2012
Republic of North Macedonia	1973	
Romania	1967	
Russian Federation		2012
San Marino		1963
Serbia	1973	
Slovakia		1963
Slovenia	1973	
Spain	1964	
Sweden		1963
Switzerland		1963
Turkmenistan	2010	
Ukraine		2012
United Kingdom of Great Britain and Northern Ireland		1963
Uzbekistan	2018	
AMERICAS		
Antigua and Barbuda		2012
Argentina	2019	
Bahamas		2012
Barbados		1968
Canada		1965
Chile		1968
Cuba	1973	
Dominica	1966	

El Salvador	2021	
Grenada	1962	
Jamaica	1966	
Paraguay	2018	
Saint Kitts and Nevis		2012
Saint Lucia	1962	
Saint Vincent and the Grenadines		2012
Trinidad and Tobago	1965	
United States of America	1970	
Uruguay		2012
SOUTH-EAST ASIA		
Maldives	2015	
Sri Lanka	2016	
WESTERN PACIFIC		
Australia	1981	
Brunei Darussalam	1987	
China	2021	
Cook Islands		1963
Fiji		1963
Japan		2012
Kiribati		2012
Marshall Islands		1963
Micronesia		1963
Mongolia		1963
Nauru		1963
New Zealand		1963
Niue		1963
Palau		1963
Samoa		1963
Singapore	1982	
Tonga		1963
Tuvalu		2012

Last update: 30 June 2021 (Source: WHO, Global Malaria Programme)

Annex – 2: Proforma for malaria epidemic/outbreak investigations

A simplified guide is proposed supported by proformas for convenience of researchers which can be used for investigating malaria outbreak/epidemic helping make informed decision by the control programme for containment and prevention of spread. The proposed format is not exclusive and can be suitably amended as per specific requirements/ operational feasibility.

1. Basic information

The first and foremost step should be to collect basic demographic information of the affected PHC/Block/ district.

State	:		District	:	
Block/PHC	:		Terrain	:	Foothill/ plain/Tribal
Population affected (%)	:		Month/year	:	
Housing condition	:	Hut/RCC/Mix	Distance to nearest healthcare facility	:	
Ecotype	:	Urban/Rural/Forest/Cross-border/Tribal			
Geographical Map	:	Include map of state showing affected district/PHC/villages			

2. Epidemiological data of affected Block PHC/district (preceding 3 years)

This information is important to ascertain parasite load in the communities and relative abundance of parasite species. Data for the preceding 3 - 5 years would be desirable*.

Year	No. blood-smears examined	ABER (%)	No. +ve cases for malaria	Parasite rate (%)	No. +ve for *Plasmodium falciparum*	% Pf of total +ve cases	Annual parasite incidence (API)	No. of deaths

*Based on state surveillance data

3. Entomological information (if available)

This information is usually not available at the block/PHC level (alternatively should be generated), which is considered important to tailor species-specific intervention strategy.

Mosquito vector species, if known	
Insecticide susceptibility status	
Larval breeding sources	

4. Seasonal distribution of cases and meteorological data of the preceding year updated to month of investigation

Rainfall data can be accessed from the nearest India meteorological centre which are considered pertinent to ascertain seasonal abundance of parasite species.

Month/ year	No. of malaria +ve cases*		Rainfall (mm)	No. of rainy days	Temperature (^{0}C)		Average Relative Humidity (%RH)
	Pf	Pv			Max	Min	
January							
February							
March							
April							
May							
June							
July							
August							
September							
October							
November							
December							

Based on state surveillance data; Pf = *Plasmodium falciparum*, Pv = *Plasmodium vivax

5. Report of insecticide spray coverage in Block/PHC

It would be desirable to have spray coverage/intervention data for the preceding 3 - 5 years. Generally, two rounds of residual insecticide (DDT/Malathion/Pyrethroid) spray are scheduled at specified intervals, yet it would be meaningful to have exact periods*

Year	Insecticide spray round	Dates of spray operation		Population coverage	
		From	To	Target pop.	% Coverage
2020	Ist				
	2nd				
2021	Ist				
	2nd				
2022	Ist				
	2nd				

***Based on state record**

6. Current observations

These are actual data to be collected by the investigating team during the study period at a given location.

6.1 Epidemiological observations

6.1.1 Passive case detection

While it would be desirable to establish malaria clinic to ensure case detection and prompt treatment to reduce morbidity and prevent mortality, mass blood surveys should also be conducted prioritizing high-risk villages to ascertain parasite load.

Malaria clinic data may be tabulated as under(the first entry is made as demonstrative exercise).

Age group in years*	**No. blood smears examined for malaria parasite**	**No. (%) blood smears positive for malaria****	**No. *Plasmodium falciparum* cases*****		**% *Plasmodium falciparum* of total +ve cases**
			Pfr	Pfg	
< 1	24	4 (17)	1	0	25
1 - <5					
5 - 15					
> 15					
Total					

*Study period: ……., ** includes both *Plasmodium vivax* and *P. falciparum* cases, *** Pfr denote *Plasmodium falciparum* with ring stage; Pfg denote both ring + gametocytes (the prevalence of gametocytes indicates delayed treatment and/or drug failure).Malaria positivity in infants (<1 year of age) indicates ongoing active transmission.

6.1.2 Mass Blood Surveys

Mass drug treatment would help drive down the transmission. Record of cross-sectional malaria prevalence surveys may be tabulated as under(first entry is made as demonstrative exercise).

Study location (Sub-division)	**Population surveyed**	**Study period**	**Type of collection**	**No. of blood - smears examined**	**Fever rate (%)**	**No. positive for malaria parasite**		
						Plasmodium falciparum	***Plasmodium vivax***	**Total cases (% smear +ve)**
Manubankul (Sabroom)	2860	June – July 2012	Afebrile	561	12	5	1	6 (1)
			Febrile	76		31	1	32 (42)
All sites		Total	Afebrile					
			Febrile					

6.2 Entomological observations

Entomological observations are central to formulate anti-vector containment measures (the first entry is made as demonstrative exercise).

6.2.1 Relative abundance of *Anopheles* mosquito species

Anopheles mosquito species	Day-resting (Human dwellings Indoor)		Cattle-biting evening collections mosquito density per person hour	CDC Trap (No. Trap nights)		Dusk-to-dawn mosquito landing catch. Mean mosquito landing rate per person night (No. of nights)	
	Mosquito density*	Total catch		Outdoor (5)	Indoor (4)	Outdoor	Indoor
An. minimus	2	3	1	2	1	1 (1)	1 (1)

*Mosquito density per person hour. Note: Abundance of vector species in human dwellings indoor suggests poor spray application of residual spraying

6.2.2 Dissection records of *Anopheles* mosquito species: First entry made as demonstrative exercise

Anopheles mosquito species	Abdominal condition*				Total mosquitoes dissected	Parity**					Vector incrimination		
	UF	FF	SG	G		NP	1 P	2 P	3 P	4 P	No. Dissected	No. gland positive	Infection rate (%)
An. minimus	0	0	9	4	13	2	6	4	1	0	332	11	3.31

*UF = Unfed, FF = Fully fed, SG = Semi-gravid, G = Gravid **P = Parity (number of oviposition cycles), NP = non-parous, 1P = Uniparous. Note: Parity suggests longevity of adult females; parity ≥2in vector mosquito indicates role in transmission supporting sporogony

6.2.3 Larval breeding habitats of *Anopheles* mosquito species (include some pics of vector breeding sites). First entry made as demonstrative exercise

Anopheles mosquito species	Ponds	Wells	Ditches	Streams	Pits	Paddy fields					
						Barren	Saplings	30 cms	>30 cms	Ready to harvest	After harvest
An. minimus	-	-	-	+	-	-	-	-	-	-	-

*(+) denotes presence of larval breeding in the given habitat

6.2.4 Insecticide susceptibility status of mosquito vector species (follow WHO standard protocol). First entry made as demonstrative exercise

Location Block, District	Mosquito species	Study period	Insecticide (Diagnostic conc.)	No. mosquitoes exposed	No. mosquitoes dead post 24 hrs exposure	Mortality (%)	Status*
Paneery, Udalguri		May 2011	DDT (4%)	35	14	40	R
			Malathion (5%)	20	18	90	VR
			Pyrethroid				
			DDT (4%)				
			Malathion (5%)				
			Pyrethroid				

*R = Resistant (<90% mortality), VR = Possible resistance (90 – 97% mortality)

6.2.5 Human-bait mosquito landing rate of *Anopheles* species in study villages (data on mosquito biting rhythm is important to devise suitable interventions).First entry made as demonstrative exercise

Location, District, State (Study period)	*Anopheles* mosquito species	Average number of mosquitoes collected per person during hours of										Mean mosquito landing rate per person night
		18-19	19-20	20-21	21-22	22-23	23-24	00-01	01-02	02-03	03-04	
Kamrup	*An. minimus*	0	0	0	1	3	4	5	2	1	0	16

6.2.6 Relative abundance of *Anopheles* mosquitospecies, sporozoite infectivity, mosquito biting and entomological inoculation rates (EIRs), and prevalence of malaria (first entry made as demonstrative exercise)

District	Study Period	Daytime resting mosquito density per person hour (MHD)	Mosquito biting/ rate/person/night (MBR)	No. mosquitoes dissected (sporozoite infection rate)	EIR (MBR x sporozoite rate) per person/ night*	% Positivity in clinical cases of malaria	
						Plasmodium falciparum	*P. vivax*
Kamrup	Jun. - Oct. 1988	07.00	14.00	332 (0.033)	0.46	17.0	11.0

*EIR = Entomological inoculation rate (mosquito biting rate x sporozoite infection rate) indicates intensity of transmission. Note: sporozoite infection rate of 0.033 is the same as 3.3%. EIR of <1 is suggestive of low-to-moderate levels of transmission. MHD denotes mosquito density per person hour

6.2.7 Risk factors of malaria

(Identification of risk factors would help prioritizing interventions. Note: given data set is merely an example; more risk factors, viz., forested villages, villages located near to the international border, access to healthcare facility by motorable road etc. can also be considered by summing up malaria cases per given period in terms of population/year). First entry made as demonstrative exercise.

Risk Factor	Population*	No. of fever cases**	Parasite rate (%)	Mean API***	Relative risk	95% confidence interval	p- value
BREADING HABITAT							
> 1 km	8057	2759	14. 71	16.79	1****	-	-
<1 km	14458	23606	32. 28	175.70	10.46	3.58 -- 30.54	<. 0001
LOCATION OF HUMAN HABITATION							
Plain	12866						
Foothills	9649						
ACCESS TO HEALTHCARE FACILITY							
< 5 km	14742						
> 5 km	7773						

* Data based on active fever surveillance in the given population category
** Total number of fever cases examined for malaria parasite during the years 1990 -1992
*** API denotes mean number of malaria cases per thousand of population per year
**** Reference category

7. Therapeutic efficacy investigations of anti-malarial drugs

Outbreak sites/locations with high positivity are ideal places to ascertain treatment cure rate of drug-regimen in force. It is usually based on extended follow up investigations for minimum up to 28 days or preferably 42 days or even longer. Alternatively, short 7-day follow up can provide preliminary idea of circulating drug-resistant strains making a strong case for extended follow up investigations.

Therapeutic efficacy status of artemisinin-based combination therapy for treatment of *Plasmodium falciparum* malaria based on 7-day follow-up study. First two entries made as demonstrative exercise.

Case No*	Age/ sex	Parasite density per cubic microlitre of blood on follow up day of					Status**
		Day 0	Day 1	Day 2	Day 3	Day 7	
01	10 F	6640	Neg	Neg	Neg	Neg	S
02	07 F	20800	8320	2240	10440	Referred	ETF
03							
04							
05							

*Target should be at least 50 follow up cases; *S = Susceptible; **ETF =Early treatment failure

8. **Conclusions:** The aforementioned data would certainly help reach some conclusions on the genesis of malaria outbreak.

9. **Specific observations:** Any other data observation(s) which are deemed important, viz., literacy levels, socio-economic conditions, knowledge attitude practice (KAP) studies, health seeking behaviour, clinical presentation of inpatients, days interval in self-reporting, quality spray operations, use of mosquito nets/LLINs etc. should also be included.

10. **Recommendations:** Given these set of observations, it should be possible to make specific recommendations for containment and spread of focal outbreaks. The technical report should be presented and discussed with concerned authorities for remedial action.

Annex – 3: A simplified study protocol for assessing residual bio-efficacy and durability of the field-distributed long-lasting insecticidal nets (LLINs)

Long-lasting insecticidal nets (LLINs) are proven evidence-based technology and being distributed globally for vector control. LLINs once distributed are expected to retain residual bio-efficacy for extended periods ranging from 3 - 5 years (serviceable life of net), but it would be in the interest of the control programme to monitor durability and efficacy against vector mosquito species ensuring protection uninterrupted. Towards this objective, a simplified study protocol is proposed which can be conveniently employed to assess the current residual bio-efficacy of LLIN to help making informed decisions for additional logistic supplies enabling net replacement in due time. Data may be collected under following heads in pre-tested study proformas detailed as below:

Relative abundance of vector mosquito species in study blocks/districts

First and foremost, step should be to ascertain vector density in target/high-risk villages. This exercise should preferably be a cross-sectional study undertaken during months of high transmission at multiple locations in population groups recipient of LLINs. Mosquito adult collections should be made by active searches early morning hours (06:00-08:00 h) as well as evening hours (18:00-20:00 h) in different habitats (resting populations indoor human dwellings and outdoor shelters) in each study location and data should be collated particularly with reference to vector density in the given format.

Anopheles **(An.) mosquito species**	**Study location # 1**		**Study location # 2**		**Study location # 3**	
	No. of mosquitoes collected in human dwellings indoors (person hour density)	No. of mosquitoes collected in outdoor shelters (person hour density)	No. of mosquitoes collected in human dwellings indoors (person hour density)	No. of mosquitoes collected in outdoor shelters (person hour density)	No. of mosquitoes collected in human dwellings indoors (person hour density)	No. of mosquitoes collected in outdoor shelters (person hour density)

Monitoring residual bio-efficacy

For this purpose, three LLINs (in use by the households) should be randomly drawn keeping one untreated net as control for comparative purposes. Field collected mosquito adult females of vector species should be exposed in WHO test bio-assay cones against net in hanging position for 3 (three) minutes for minimum of five replicates in batches of 10 mosquitoes for each test (10 x 5 = 50 adults). Number of mosquitoes knock-down after exposure period and dead post 24 h recovery period should be recorded as per given format.

Type of LLIN	Manufacturing date of LLIN (month of distribution)	Study location (Block/District)					
		Location # 1		Location # 2		Location # 3	
		No. & (%) mosquitoes knockdown post 3 min exposure/ total exposed[a]	No. & (%) mosquitoes dead post 24h exposure/ total exposed[b]	No. & (%) mosquitoes knock-down post 3 min exposure/ total exposed	No. & (%) mosquitoes dead post 24h exposure/ total exposed	No. & (%) mosquitoes knock-down post 3 min exposure/ total exposed	No. & (%) mosquitoes dead post 24h exposure/total exposed

[a] data based on exposure of 10 mosquitoes per cone-bioassay
[b] mosquito mortality <80% denotes depletion of residual insecticide on fibre than optimum

These test data can be supplemented by ring-net bioassay to substantiate residual bio-efficacies for which 11 mosquito adult females should be exposed in ring-net against net fibre and time required for knock-down of Ist, 6th and 11th mosquito should be recorded for maximum exposure of 1 h. Time taken for knock-down of 6th mosquito is regarded as the median knock-down time.

Type of LLIN	Manufacturing date of LLIN (month of distribution)	Study location (Block/District)								
		Study location # 1			Study location # 2			Study location # 3		
		Mosquito knock-down time in minutes (mean± SD) a			Mosquito knock-down time in minutes (mean± SD)			Mosquito knock-down time in minutes (mean± SD)		
		I^{st}	6^{thb}	11^{th}	I^{st}	6^{th}	11^{th}	I^{st}	6^{th}	11^{th}

[a] Data based on exposure of 11 mosquitoes per ring-net bioassay
[b] Median knockdown time for 6th mosquito of >10 minutes denotes significant depletion of residual insecticide on netting fibre

Net durability

Durability and physical integrity of field-distributed nets depends upon community usage (throughput year or seasonal) and washing practices (monthly, quarterly, six-monthly by soap water or any other method) which may vary between populations.For cross-sectional survey, an estimated 25% of the target population should be visited and nets inspected physically for being torn, number of holes and stitches, existing condition of net etc; and data may be tabulated in the given format.

S. N.	Question	Physical condition of		Any
		Type of LLIN N[a] =	Type of LLIN N =	
1.	No. of torn nets having holes (% of nets inspected)			
2.	Total number of holes (Number of holes/ No. of torn net)			
	Small (0.5 – <2 cm diameter)			
	Medium (2 – 10 cm diameter)			
	Large (>10 cm diameter)			
3.	Total number of holes in position (Number of holes/ No. of torn net)			
	Lower half of net			
	Upper half of net			
	Roof			
4.	Physical aspect of net (Percentage of nets inspected)			
	Clean			
	A bit dirty			
	Dirty			
	Very dirty			

[a] Number of nets inspected; Type of LLIN means brand distributed, viz., PermaNet, Olyset net, Duranet

As per WHOPES criteria, the net serviceable life for maintaining optimum residual bio-efficacy (mosquito mortality ≥80%) and integrity should be minimum of 3 (three) years of continuous use by the communities in field conditions. In context of malaria elimination, it is crucial to monitor the bio-efficacy and serviceable life of previously distributed nets to ensure continuous protection by replacing those worn-out by fresh supply. In conclusion, monitoring residual bio-efficacy of LLINs should be in-built activity of the National Malaria Control Programme making provisions for net replacement providing uninterrupted protection in place and time.

Glossary: Biological terms, Concepts and Case definitions

A

Advocacy: A continuous and adaptive process of gathering, organizing, and formulating information into argument with a view to raising resources or gaining the acceptance and commitment of political and social leadership to a development programme thereby preparing a society for its acceptance.

Allopatric speciation: It is a mode of speciation by geographical separation of two of more populations by physical barrier such as ocean resulting in reproductive isolation.

Anaemia: Reduction in the number of circulating red blood cells or in the quantity of haemoglobin. It is a common consequence of repeated episodes of malaria.

Annual Blood Examination Rate (ABER): It is a measure of disease surveillance (indicator of programme operation) calculated by number of parasitological tests undertaken per 100 people at risk per year targeted at 10% - an expected rate of fever prevalence of any cause in the community. ABER <10% per year is considered less than adequate.

***Anopheles*:** A genus of mosquito some species of which can transmit human malaria. There are about 50 anopheline mosquito species capable of transmitting malaria spread across continents.

Anthropophagic/anthropophilic mosquito: Mosquito species having strong predilection for human host for bloodmeal. Anthropophilic index(AI) refers to percentage of mosquitoes having fed on human host; an important measure having implications in disease transmission control.

Antibody (Ab): An antibody is a protein produced by the body's immune system in response to specific antigen.Each antibody contains a paratope which recognizes a specific epitope on an antigen acting like a lock and key binding mechanism.

Antigen (Ag): Any foreign body such as bacteria, viruses or parasites that stimulates production of specific antibodies as a result of immune response of the host. Each antigen has distinct surface features or epitopes resulting in specific responses.

Anti-malarial drug-resistance: Ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug in doses equal to or higher than those usually recommended but within the limits of tolerance of the subject.

Antimicrobial resistance: Antimicrobial resistance is the result of microbes changing in ways that reduce or eliminate the effectiveness of drugs, chemicals, or other agents to cure or prevent infections.

Apical membrane antigen 1 (AMA1): It is an asexual blood-stage protein expressed in the

invasive merozoite form of *Plasmodia* species which are the causative agent of malaria. The antigen is designated AMA-1 by virtue of appearing to be located in the apical complex and appears to be transported to the merozoite surface close to the time of schizont rupture.

Aptamer: Aptamers are oligonucleotide or peptide molecules that bind to a specific target molecule. Aptamers are usually created by selecting them from a large random sequence pool, but natural aptamers also exist in riboswitches. Aptamers can be used for both basic research and clinical purposes as macromolecular drugs.

Artemisinin: A class of drugs used for the treatment of malaria (usually as a part of a combination therapy) derived from the sweet wormwood (*Artemisia annua*).

Asymptomatic malaria: *Plasmodium* infections that do not lead to clinical symptoms and therefore remain undetected by fever-based surveillance systems. Asymptomatic cases occur without eventual overt symptoms and do not come to clinical attention, thus representing a largely hidden reservoir.

Atovaquone: A drug used for treating malaria. It is often used in combination (atovaquone-proguanil) which can be used for both prevention and treatment.

Autochthonous: It refers to local transmission, which can either be indigenous (a geographic area where malaria occurs regularly) or introduced (in a geographic area where malaria does not occur regularly).

B

B-cell (B-lymphocyte): White blood cells of the immune system that are derived from the bone marrow and spleen. B cells develop into plasma cells, which produce antibodies.

Behavioural resistance: It is attributed to innate abilities of mosquito species to detect and avoid contact with insecticide treated/sprayed surfaces due to repellency/excito-repellency or irritability. It is commonly observed in *Anopheles minimus* populations specific to North-East India resulting in change of resting behaviour from indoors to outdoors.

Benign tertian malaria (BT malaria): Malaria caused by *Plasmodium vivax* that is an acute self-limiting febrile illness with fever spikes at 48 hours interval and no/rare complications or death. Therefore, the illness caused by this parasite termed as benign tertian malaria.

Biological Control: It is an integral component of integrated vector control strategy defined as reduction of pest population by natural enemies/predators such as by application of larvivorous fish (Guppy or Gambusia) for control of mosquito larval breeding.

Biotope: A biotope is an area (ecological niche) of uniform environmental conditions providing an ideal living habitat for a specific community/population.

C

Carbamate: A class of chemical product used as insecticide for population control.

Cerebral malaria: A severe malaria syndrome in which infected red blood cells obstruct blood circulation in the small blood vessels in the brain and/or release cytokines that disrupt normal brain function.

Chemoprophylaxis: The use of antimalarial drugs to prevent malaria disease.

Chloroquine: A drug used against malaria for both prevention and treatment. It is very safe and inexpensive drug, but its value has been compromised by the emergence of chloroquine-resistant malaria parasites.

Cinchonism: Side effects from quinine or quinidine. Includes tinnitus, headache, nausea, diarrhoea, altered auditory acuity, and blurred vision. The term derives from cinchona bark, the natural source of quinine.

Climate change: Climate change refers to a large-scale long-term shift in the planet's weather patterns and average temperatures attributed largely to the increased levels of atmospheric carbon dioxide produced using fossil fuels/increased anthropogenic activities.

Clinical cure: Elimination of malaria symptoms sometimes without eliminating all parasites.

Coma: A decreased state of consciousness from which a person cannot be roused.

Community: A group of people with common interests and fellowship living in the same area, i.e., more intimately involved than at either district or regional areas.

Community-based Organization (CBO): Local organization functioning at grassroots level such as Faith-based organizations (FBO).

Community ownership: Community participation that has been developed to increased people's sense of control over issues that affect their lives.

Community's own resource persons: Trained health workers such as Community Health Workers, Voluntary Health Workers, Village Link Workers who are members of the community where they work, selected by the community, answerable to the community for their activities, supported by the community as well as the health system having a short-term training than professional health workers.

Community participation: The active involvement of people living together in some form of social organization and cohesion in the planning, operations and evaluation of a programme using local, national, and other resources. It is a process through which communities ultimately influence and share control over the development, local initiatives, decisions, and resources of projects, e.g., malaria control that directly affect them.

Communication strategy: A plan to communicate health messages using methods, e.g., pamphlets, presentations, posters, workshops, films, videotape, or slide shows, village street drama, radio spots, TV spots, newspapers that are appropriate to the target population.

Congenital malaria: Malaria in a new-born or infant transmitted from the mother.

Cost of illness: Many reports use expenditures on medical care (actual money spent) as the cost of illness. Ideally, the cost of illness should also take into account factors that are more difficult to measure, such as work-related costs, educational costs, the cost of support services required by the medical condition, and the amount individuals would pay to avoid health risks.

Cytoadherence: It is the property of *Plasmodium falciparum* infected RBC to adhere to various host cell types such as endothelial cells and uninfected red blood cells causing the parasite to sequester in deep vascular beds and avoid splenic clearance. Cytoadherence and sequestration are distinct features of *P. falciparum* resulting in severe manifestations, viz., cerebral malaria, coma.

D

DALY (Disability-Adjusted Life Year): A summary measure of the health of a population. One DALY represents one lost year of healthy life and is used to estimate the gap between the current health of a population and an ideal situation in which everyone in that population would live into old age in full health.

DEET: N,N-diethyl-meta-toluamide, an ingredient of insect repellents.

Deltamethrin: An insecticide belonging to class Pyrethroids.

Disease burden: The total significance of disease for society beyond the immediate cost of treatment. It is measured in years of life lost to ill health or the difference between total life expectancy and disability-adjusted life expectancy (DALY).

Diurnal: Relates to daytime activity of biological species (opposite: nocturnal: night-time activity), viz., dengue mosquito is day-time biter opposed to malaria transmitting mosquito biting during the night.

DNA barcoding: It is a method of species identification using a short section of DNA from a specific gene or genes. The premise of DNA barcoding is that, by comparison with a reference library of such DNA sections (also called "sequences"), an individual sequence can be used to uniquely identify an organism to species. The most commonly used barcode region for animals is a portion of the cytochrome c oxidase I (COX1) gene also known as MT-COI, found in mitochondrial DNA.

DNA fingerprinting: Also called DNA profiling, it is the process of determining an individual's DNA characteristics. It is a forensic technique used in criminal investigations, parentage testing and genealogical and medical research.

Drug resistance: Defined as ability of the parasite strain to survive or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the tolerance of the subject. In malaria, drug resistance results in delay or failure to clear asexual parasites from the blood, which allows production of the gametocytes that are responsible for transmission of the resistant genotype.

E

Early Treatment Failure (ETF): Defined as condition meeting any one of the following criteria: (i) danger sign or severe malaria on day 1, 2 or 3 in presence of parasitaemia; (ii) parasitaemia on day 2 higher than day 0 irrespective of axillary temperature; (iii) parasitaemia on day 3 with axillary temperature ≥37.50C; (iv) parasitaemia on day 3 ≥ 25% of count on day 0.

Ecology: It is the study of inter-relationships between living organisms and their physical environment.

ELISA: Enzyme-linked immunosorbent assay. This laboratory test is often used to determine whether mosquito salivary glands are positive for sporozoites.

El Niño-Southern Oscillation (ENSO): It is an irregular periodic variation in winds and sea surface temperatures over the tropical eastern Pacific Ocean affecting the climate of much of the tropics and subtropics. The warming phase of the sea temperature is known as El Niño and the cooling phase as La Niña.

Endemic: It refers to the constant presence and/or usual prevalence of a disease or infectious agent in a population within a geographic area. Hyperendemic refers to persistent high levels of disease occurrence vis-à-vis meso-endemic with low-to-medium levels of transmission.

Endophilic mosquito: A mosquito species that primarily rests indoors, inside a human dwelling, during the period between the end of blood-feeding and the onset of searching for an oviposition site.

Endophagic mosquito: An endophagic mosquito is a mosquito that feeds indoors.

Entomological Inoculation rate (EIR): It is a metric of malaria transmission intensity estimating the risk of contracting malaria in a particular area by expressing the number of infective bites per person calculated multiplying mosquito biting rate per person night (MBR) x sporozoite infection rate.

Epidemic: Sudden increase of cases of a disease what is normally expected to occur in a population of given area/territory. Outbreak carries the same definition of epidemic, but it is more often focal in nature affecting limited geographic area. Pandemic instead refers to disease when spread over continents affecting world's population at large at given point of time.

Epidemiology: A study (scientific, systematic, and data-driven) of the distribution (frequency, pattern) and determinants (causes, risk factors) of health-related states and events in specified populations (neighbourhood, school, city, state, country, global). It is a cornerstone of public health that helps shape policy decisions and evidence-based practice by identifying risk factors for disease and targets for preventive healthcare.

Etiology: The cause or origin of a disease or disorder; the study of the factors that cause disease and of the method of their introduction into the host.

Erythrocytic stage: A stage in the life cycle of the malaria parasite found in the red blood cells. Erythrocytic stage parasites cause the symptoms of malaria.

Ex-erythrocytic stage: A stage in the life cycle of the malaria parasite found in liver cells (hepatocytes). Exoerythrocytic stage parasites do not cause symptoms.

Exophagic mosquito: An exophagic mosquito is a mosquito that feeds outdoors.

Exophilic mosquito: A characteristic of mosquito species having preferences for resting outdoors human dwellings between two consecutive bloodmeals. Residual insecticides in house dwellings are less effective at controlling exophilic mosquitoes.

F

***falciparum*:** A malaria parasite species of *Plasmodium* infecting humans.

Fansidar: Brand name of sulfadoxine-pyrimethamine, a drug used for treatment of malaria. Its value has been compromised by the emergence of drug-resistant malaria parasites.

G

G6PD deficiency: An inherited abnormality that causes the loss of red blood cell enzyme. Primaquine should not be administered to G6PD deficient subjects, infants as well as in pregnancy for its severity causing RBC haemolysis.

Gametocyte: The sexual stage of malaria parasites. Male gametocytes (microgametocytes) and female gametocytes (macrogametocytes) are inside red blood cells in the circulation. If they are ingested by a female *Anopheles* mosquito, they undergo sexual reproduction which starts the extrinsic (sporogony) cycle of the parasite in the mosquito (definitive host). Gametocytes of *Plasmodium falciparum* are typically banana or crescent-shaped.

Gene: The basic unit of inheritance. A gene is a segment of DNA that specifies the structure of a protein or an RNA molecule.

Genetic control: For genetic control, the modified pest becomes a biocontrol agent acting via mating. This leads to a very high degree of species specificity for population control. Furthermore, the genetically modified mosquitoes actively disperse and seek out conspecific mates.

Global health: An area for study, research, and practice that places a priority on improving health and achieving health equity for all people worldwide.

Greenhouse effect: Warming of Earth's surface and troposphere (the lowest layer of the atmosphere) caused by the presence of water vapour, carbon dioxide, methane, and certain other gases in the air.

H

Haemoglobin: The red oxygen-carrying protein found in red blood cells (RBCs)

Haemolysis: Destruction of red blood cells. Malaria causes haemolysis when the parasites rupture the red blood cells in which they have grown.

Hepatocytes: Liver cells

Hepatomegaly: Enlarged liver.

Herd immunity: It refers to resistance to spread of an infectious disease within a population based on pre-existing immunity in high proportion of individuals as a result of previous infection or vaccination.

Hypnozoite: Dormant form of malaria parasites found in liver cells. Hypnozoites occur only with *Plasmodium vivax* and *P. ovale*. After sporozoites (inoculated by the mosquito) invade liver cells, some sporozoites develop into dormant forms (the hypnozoites), which do not cause any symptoms. Hypnozoites can become activated months or years after the initial infection producing relapse.

Hypoglycaemia: Low blood glucose level. Hypoglycaemia can occur in malaria. In addition, treatment with quinine and quinidine stimulate insulin secretion reducing blood glucose level.

I

Immunity: Protection generated by the body's immune system in response to previous malaria attacks resulting in ability to control or lessen severity due to malaria attack.

Imported malaria: Malaria acquired outside a specific geographic area.

Incidence: The number of new cases of a disease or disorder in defined population over a period of time, e.g., Annual Parasite Incidence (API) means number of cases in a year per unit population generally described per thousand, viz., API 2 means 2 cases per thousand population in a given year considered a sensitive indicator mandating application of interventions in a given area.

Incubation period: The interval of time between infection by a microorganism and the onset of the illness or the first symptoms of the illness. In malaria, the incubation is between the mosquito bite and the onset of symptoms that varies between parasite species usually between 10 to 12 days post exposure.

Indoor residual spraying (IRS): Treatment of houses where people spend night-time hours by spraying insecticides that have residual efficacy (that continue to affect mosquitoes for several months). Residual insecticide spraying aims to kills mosquitoes when they come to rest on the walls usually after blood meal.

Induced malaria: Malaria acquired through artificial means, e.g., blood transfusion, shared needles or syringes.

Insecticide resistance: It is defined as the ability of an insect to withstand the effects of an insecticide by becoming resistant to its toxic effects by means of natural selection and mutations.One or more physiological mechanisms may be involved including behavioural resistance, reduced penetration, alteration of target site nerve receptors (kdr, Rdl and Ace), and metabolic resistance (biochemical resistance).

Internal transcribed spacer 2 (ITS 2): It is the spacer DNA situated between 5.8S small-subunit ribosomal RNA (rRNA) and 28S large-subunit rRNA genes in the chromosome or the corresponding transcribed region in the polycistronic rRNA precursor transcript.

Introduced malaria: Mosquito-borne transmission of malaria from an imported case in a geographic area where malaria does not occur regularly.

J

Jaundice: Yellow discoloration of skin and eyes due to elevated blood levels of bilirubin. It indicates a problem with the liver or bile duct. It is of common occurrence in malaria-endemic populations due to repeated exposures.

K

***kelch*13-propeller gene:** Polymorphism in portions of *Plasmodium falciparum* gene encoding *kelch*(K13)-propeller domains has been associated with delayed parasite clearance after treatment with artemisinin-based combination therapy.

Key informants: Persons carefully selected to inform the programme because of their special knowledge of relevant aspects of the target population or in-depth understanding of the key-issues.

***knowlesi*:** A malaria parasite species of *Plasmodium* normally infecting monkeys, yet capable of infecting humans.

L

La Niña: It is an irregularly recurring upwelling of unusually cold water to the ocean surface along the western coast of South America that often occurs following an El Niño and that disrupts typical regional and global weather patterns especially in a manner opposite to that of El Niño.

Late Treatment Failure (LTF): It is further categorized into (1) late clinical failure characterized by danger sign or severe malaria in presence of parasitaemia on any day between day 4 and day 28 (day 42) with axillary temperature $\geq 37.5^{0}$C; (2) late parasitological failure categorized by presence of parasitaemia on any day between 7 and day 28 (day 42)

with axillary temperature <37.5^{0}C in patients who did not meet any of the criteria of ETF in either category.

Leukocyte: White blood cell (WBC).

Leukopenia: Decrease in total white blood cell count.

Lymphocytes: Lymphocytes are white blood cells that are also one of the body's main types of immune cells made in the bone marrow and found in the blood and lymph tissue. There are two categories of lymphocytes known as B lymphocytes and T lymphocytes commonly referred to as B cells and T cells.

M

Macrogametocyte: The female form of the gametocyte.

***malariae*:** A malaria parasite species of *Plasmodium* infecting humans responsible for Quartan malaria characterized by fever spike at 72 hours intervals.

Malaria antigen detection tests kits: These are group of commercially available rapid diagnostic tests (RDTs) that allow quick diagnosis of malaria by people who are not otherwise skilled in traditional laboratory techniques for diagnosing malaria or in situations where such equipment is not available, e.g., kits based on detection of Histidine Rich Protein 2 (HRP 2) an antigen specific for *Plasmodium falciparum* secreted from erythrocytes infected with rings, trophozoites, schizonts and immature gametocytes.

Malaria elimination: Interruption of indigenous transmission that is reducing the rate of malaria cases to zero of a specified parasite species in a defined geographic area. Continued intervention measures are required to prevent the re-establishment of transmission.

Malaria eradication: Permanent reduction to zero of the worldwide incidences of infection caused by human malaria parasites as a result of deliberate efforts. Once eradication has been achieved, intervention measures would no longer be needed.

Malignant Tertian malaria (MT malaria): Malaria caused by *Plasmodium falciparum* characterized by intense paroxysms with acute cerebral, renal, or gastrointestinal manifestations in severe cases.

Merozoite: It is a developmental stage in malaria parasite cycle produced asexually by multiple fission of schizonts in liver cells that invade human RBCs. Merozoites multiply further in RBCs invading other RBCs resulting in clinical paroxysm.

Microgametocyte: The male form of the gametocyte.

Molecular taxonomy: It is a significant development in insect systematics that permits an exact and rapid method of distinguishing specimens based on their interspecific variations. Molecular techniques based on PCR applications have helped to establish genetic relationship between the members of different taxonomic categories.

Monocyte: Leukocyte with a large, usually kidney-shaped nucleus. Within tissues,

monocytes develop into macrophages which ingest bacteria, dead cells, and other debris.

Mosquito biting rate (MBR): Number of mosquitos collected landing on human host per person per night during all night catch (dusk-to-dawn). It can similarly be calculated per person hour of the night or monthly and annually. These data are of epidemiological significance giving information on biting behaviour of mosquito species and transmission intensities helping institute control interventions.

Mosquito parity: It is defined as the number of times a female mosquito has laid eggs determined by dilatations on ovaries, viz., one dilatation means uniparous, two dilatations equal biparous and no dilation means nulliparous. In theory, the number of dilatations in the ovarioles (follicles) must equal to the number of egg-laying cycles in female mosquitoes.

Multidrug resistance: When parasite is resistant to more than two operational antimalarial compounds of different chemical classes and modes of action.

Multiplex PCR: It refers to the use of polymerase chain reaction to amplify several DNA sequences simultaneously. This process amplifies DNA in samples using multiple primers and a temperature-mediated DNA polymerase in a thermal cycler.

N

Niche: An ideal ecological habitat most suitable for biological species for survival and proliferation.

Non-Government Organization (NGO): A Non-Governmental Organization (NGO) or Civil Society Organization is any organization not established by the Government. It is a non-profit group that functions independently of any Government. These comprise the "third sector" of modern society in addition to the public and private sectors.

O

One health: It is a collaborative, multisectoral, and transdisciplinary approach working at the local, regional, national, and global levels with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environment.

Oocyst: A cyst containing a zygote formed by a malaria parasite. Ookinete migrate through the gut wall of the mosquito host and forms the oocyst on the epithelium and produce sporozoites by fission.

Ookinete: The motile zygote of the malarial parasite formed by the fusion of female and male gametes (the only diploid stage of malaria parasite) that penetrates the mosquito (the definitive host) stomach to form an oocyst under the outer gut lining.

ovale: A parasite species of *Plasmodium* infecting humans commonly found in Africa.

P

Parapatric speciation: It is a mode of speciation when new species evolve in contiguous, yet spatially segregated habitats. Unlike allopatric speciation, the populations that are diverging during parapatric speciation maintain a zone of contact and do not cease the exchange of genes completely.

Parasite: An organism that lives in/on an organism of another species (its host) and benefits by deriving nutrients at the other's expense. For example. human malaria parasite is a unicellular protozoan that inhabits RBCs and multiplies asexually devouring on haemoglobin.

Parasitaemia: The presence of parasites in the blood. The term can also be used to express the quantity of parasites in the blood usually by density per cubic microlitre (µl).

Paroxysm: A sudden attack or increase in intensity of a clinical symptoms usually occurring in periodical intervals.

Pernicious malaria: It is a grave form of chronic malaria caused by *Plasmodium falciparum* characterized by severe malarial paroxysms/life threatening illness affecting vital organs.

Phagocyte: A type of white blood cell that can engulf and destroy foreign organisms, cells and particles. Phagocytes are an important part of the immune system.

Platelets: Platelets, or thrombocytes are small,colourless cell fragments in our blood that form clots and stop or prevent bleeding. Platelets are made in our bone marrow, the sponge-like tissue inside our bones. Bone marrow contains stem cells that develop into red blood cells, white blood cells, and platelets.

***Plasmodium*:** The genus of the parasite that causes malaria. The genus includes many species. The four species that naturally infect humans are *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale* and *P. malariae. Plasmodium knowlesi*, instead is a zoonotic species that naturally infects macaques in Southeast Asia that can also infect humans.

Polymerase chain reaction (PCR): It is a laboratory technique used to amplify DNA sequences. The method involves using short DNA sequences called primers to select the portion of the genome to be amplified. The temperature of the sample is repeatedly raised and lowered to help a DNA replication enzyme copy the target DNA sequence. The technique can produce a billion copies of the target sequence in just a few hours.

Polymorphic: Literally meaning having more than one form. In terms of genes, it means that there are several variants (alleles) of a particular gene that occur simultaneously in a population.

Presumptive treatment: Treatment of clinically suspected cases without or prior to results from confirmatory laboratory tests.

Prevalence: The number of existing cases of a disease in a population at a given point of time, viz., parasite infection rate described in terms of number of positive cases per hundred subjects examined. In malariology, parasite rate of 10% means 10 confirmed positive cases per 100 subjects examined in a given population. Abundance of *Plasmodium falciparum*

cases (% Pf) calculated as proportion of Pf cases of total positive cases is a strong indicator for intensification of control interventions for its clinical severity and fatal outcome (if not treated in time).

Primaquine: An anti-relapse drug used against malaria for the prevention of *P. vivax* or for the eradication of the hypnozoites of *P. vivax* and *P. ovale*.

Protozoan: Single-celled organism that can perform all necessary functions of metabolism and reproduction. Some protozoa are free-living, while others, including malaria parasites, parasitize other organisms for their nutrients and life cycle.

Public Private Partnership: A Public-Private Partnership (PPP) is defined as a long-term contract between a private party and a government agency providing public service in which the private party bears significant risk and management responsibility.

Pyrethroid: A class of insecticides derived from the natural pyrethrin.

Q

Quartan malaria: Malaria caused by *Plasmodium malariae*. Quartan fever is a form of malaria where an onset of fever occurs in an interval of three to four days (72 hours interval), hence the name "quartan."

Quinine: A drug used for treatment of malaria obtained from the bark of the cinchona tree. Quinine is used for treatment but not prevention of malaria.

Quotidian malaria: Malaria caused by *Plasmodium knowlesi* that tends to have fever spike every 24 hours and is therefore often called daily or "quotidian" malaria. Uncomplicated *P. knowlesi* malaria can be treated with antimalarial drugs. At least 10% of people infected with *P. knowlesi* develop severe malaria.

R

Radical cure: (also: radical treatment) Complete elimination of malaria parasites from the body; the term applies specifically to elimination of dormant liver stage parasites (hypnozoites) found in *Plasmodium vivax* and *P. ovale*.

Randomly amplified polymorphic DNA (RAPD): It is a PCR-based technique which uses arbitrary primers which bind to the nonspecific sites on the DNA and amplify the DNA. These amplified fragments are then migrated on agarose gel and difference in the band pattern is observed to discriminate biological species.

Recombinant DNA (rDNA): It is a technology that uses enzymes to cut and paste together DNA sequences of interest. The recombined DNA sequences can be placed into vehicles called vectors that ferry the DNA into a suitable host cell where it can be copied or expressed.

Recrudescence: It is the term for recurrence of infection with all malaria species including *Plasmodium falciparum, P. malariae* and *P. knowlesi*, which lack hypnozoites. This occurs when the infection (unless a new infection) has persisted in the blood at undetectable levels and then becomes detectable again.

Relapse: Recurrence of disease after it has been apparently cured. In malaria, true relapses are caused by reactivation of dormant liver stage parasites (hypnozoites) found in *Plasmodium vivax* and *P. ovale*.

Resistance: The ability of an organism to develop ways to be impervious to specific threats to their existence. The malaria parasite has developed strains that are resistant to drugs such as chloroquine. The *Anopheles* mosquito has developed strains that are resistant to DDT and other insecticides.

Restriction fragment length polymorphism (RFLP): These are differences among individuals in the lengths of DNA fragments cut by enzymes. Restriction enzymes are proteins that cut DNA at short, specific sequences called restriction sites. After a segment of DNA has been cut into pieces with restriction enzymes, researchers can examine the fragments using a laboratory method called gel electrophoresis, which separates DNA fragments according to their size. If two individuals have differences in their DNA sequences at particular restriction sites, then the restriction enzymes will cut their DNA into fragments of different lengths thus help establish phylogenetic relationships.

Reticulocytes: Red blood cells that are still developing. They are also known as immature red blood cells made in the bone marrow and sent into the bloodstream. About two days they develop into mature red blood cells. *P. vivax* infects only reticulocytes thus self-limiting infection.

Ribosomal DNA (rDNA): It is the sequence of DNA that codes for ribosomal RNA. In eukaryotes, the ribosomal DNA contains a tandem repeat of a unit segment, an operon, composed of NTS, ETS, 18S, ITS1, 5.8S, ITS2, and 28S tracts, as well as a gene coding for 5S ribosomal RNA.

RTS,S/AS01 (RTS,S): It is a first generation vaccine targeted against *Plasmodium falciparum*, which is shown to provide partial protection against malaria in young children.

S

Schizogony: It relates to asexual reproduction of parasite by multiple fission within host RBC resulting in production of merozoites.

Schizont: A developmental form of the malaria parasite that contains many merozoites. Schizonts are seen in the liver-stage and blood-stage parasites.

Sequelae: Morbid conditions following as a consequence of a disease.

Serology: The branch of science dealing with the measurement and characterization of antibodies and other immunological substances in body fluids, particularly serum.

Sibling-species: Two or more biological species/populations which are often indistinguishable and are completely or partially reproductively isolated (also referred as cryptic-species), but can be characterized by molecular tools, viz., PCR applications. Group of similar such species constitute what is called species complex, e.g., *Anopheles culicifacies* complex.

Social marketing: An approach using marketing techniques to promote and distribute socially beneficial interventions rather than commercial products.

Social mobilization: A process of bringing together all interested intersectoral partners and allies to determine felt needs and raise awareness of and demand for a particular development objective. It involves enlisting the support of all stakeholders, including institutions, groups, and communities, in identifying, raising and managing human and material resources, thereby increasing and strengthening participation for self-reliance and sustainability of achievements.

Species: Group of individuals which are reproductively isolated from other such groups in the same genus that have similar characteristics.

Speciation: It is an evolutionary process of the formation of new and distinct species by genetic differentiation. The new species are reproductively isolated from the ancestral species.

Species group: Group of species which are very closely related but can be distinguished morphologically as well as by molecular assays unequivocally.

Species-sanitation: Population control of a biological species by species-specific intervention strategies such as targeting breeding/resting habitats. It is cost effective rather than blanket coverage of interventions.

Splenomegaly: Enlargement of the spleen often found in some malaria patients. Splenomegaly can be used to measure malaria endemicity during surveys (e.g., in communities or in school children).

Splenectomy: Removal of the spleen.

Sporogony: It is process resulting in production of sporozoites (the infective stage of malaria parasite) in the mosquito host from oocysts (the only diploid state of parasite) by multiple fission.

Sporozoite: It is an infective stage in the life cycle of the malaria parasite. Sporozoites are produced in the mosquito that migrate to the mosquito's salivary glands. These are inoculated in the process of taking blood meal on human host. In the humans, the sporozoites enter liver cells where they develop into the next stage of the malaria parasite life cycle (the liver stage or ex-erythrocytic stage).

Sporozoite infection rate (%): The percentage of female anopheline mosquitoes of a particular species that bear sporozoites in their salivary glands.

Sub-patent parasitaemia: An infection detectable by molecular methods, but not by microscopy or Rapid Diagnostic Test kits also referred to as low-density infection.

Sulfadoxine-pyrimethamine: A drug used against malaria. Its value has been compromised by the emergence of drug-resistant malaria parasites.

Sympatric speciation: It is process of speciation wherein a new species evolves from a single ancestral species while inhabiting the same geographic region without geographic isolation.

T

Therapeutic efficacy (TE): It is expressed in terms of degree of benefit/relief afforded by the given drug in the recommended dose range or success rate in achieving a defined therapeutic end point. Therapeutic efficacy is assessed based on follow up of clinical as well as parasitological response for 28 days or extended follow up to 42 days and categorized as treatment success or treatment failure based on prescribed criteria.

Thrombocytopenia: Low platelet count that can lead to impaired blood clotting and spontaneous bleeding.

Tinnitus: Ringing sound/buzzing in the ears, a common side effect of quinine treatment.

Treatment failure: failure to resolve or recurrence of fever and/or parasitaemia within 28 days of initiation of treatment.It is further categorized into early treatment failure (ETF), late treatment failure (LTF) depending on certain prescribed criteria.

Treatment success: absence of parasitaemia on day 28 (day 42) irrespective of axillary temperature in patients who did not meet any of the criteria of early treatment failure, late treatment failure or late parasitological failure.

Trophozoite: It is an active developmental stage of the malaria parasite in the host RBC feeding on haemoglobin. After merozoites have invaded the red blood cell, they develop into trophozoites (sometimes, early trophozoites are called "rings" or "ring stage parasites"); trophozoites develop into schizonts.

V

Vaccine: A preparation that stimulates an immune response which can prevent an infection or create resistance to an infection.

Vector: A disease vector is any living agent that carries and transmits infectious pathogen to another living organism. Malaria is a vector-borne disease transmitted by bite of *Anopheles* mosquito to humans caused by protozoan parasite *Plasmodium* (in an infectious agent). There are more than 50 *Anopheles* mosquito species spread across continents capable of transmitting malaria parasite.

Vector bionomics: A study of biological characteristics of a mosquito species, e.g., larval breeding habitats, resting behaviour, host-feeding preferences and disease transmission relationships in the given ecological context. These data are of significance in formulating species-specific vector control strategies.

Vectorial capacity: It is a measure of transmission potential of a vector-pathogen system

involving number of variables, viz., the parasite's extrinsic incubation period (EIP, n days); the ratio of mosquitoes to humans (m); mosquito survival through one day (p); and human biting rates (a) that are taken into account to calculate estimated daily rate at which future inoculations may arise from infected case: V=ma2pn−ln(p).

Vector control: Vector control aims to limit the transmission of pathogens by reducing or eliminating human contact with the vector by interventions such as indoor residual spraying with insecticides, space spraying, biological control, source reduction and promoting allied preventive measures.

Vector density: Relative abundance of mosquitoes in given habitat described as number of mosquitoes collected per person hour.

Virus: A microorganism composed of a piece of genetic material – RNA or DNA – surrounded by a protein coat. To replicate, a virus must infect a cell and direct its cellular machinery to produce new viruses.

vivax: a malaria parasite species of the genus *Plasmodium* commonly found in South-East Asia.

Z

Zoogeography: Branch of biogeography that deals with the distribution of animal species having implication in evolution and speciation.

Zoonotic diseases: Transmission of disease of animals to human beings, viz., rabies (a disease of dogs) transmitted to humans by dog bite. Zoonotic malaria (*Plasmodium knowlesi*), an infection of monkeys is transmitted to humans by occupational exposure visiting forests inhabited by monkeys - an emerging paradigm in Southeast Asia, yet no human-to-human transmission is established.

Zoophilic/zoophagic mosquito: A biological species having strong preferences for animals than humans, e.g., mosquito species feeding on animal for bloodmeal host than human host.

Author's Corner

Having acquired higher education and professional experience at world's acclaimed institutions in India and abroad, Vas Dev worked for the ICMR – National Institute of Malaria Research (formerly Malaria research Centre), a premier research organization of the Indian Council of Medical Research. During his entire length of service (1988-2016), he served as Scientist-in-Charge of the Field Station based in Assam (North-East India) to field-test alternate interventions for malaria control that are community-based, sustainable and cost-savvy. During his stewardship, number of technologies were put to field-evaluation in high-risk areas including; (i) insecticide-treated netting materials for vector control, (ii) monitoring therapeutic efficacy of anti-malarial drugs helping upgrading drug-policy for radical cure, (iii) alternate diagnostics, (iv) and large-scale introduction of larvivorous fish promoting biological control; all these interventions were incorporated in healthcare services resulting in substantial disease transmission reduction. His research efforts resulted in-depth understanding of local disease epidemiology and vector control specific to North-East region and culminated in more than160 research publications in indexed journals. His expertise was sought both nationally and internationally and resulted in sound documentation including books: (i) 'Towards Malaria Elimination – A Leap Forward published in 2018 by IntechOpen, London (co-edited with Prof. Sylvie Manguin, Montpellier, France), and (ii) 'Vector Biology & Control: An Update for Malaria Elimination Initiative in India' commissioned by the National Academy of Sciences, India published in 2020. Vas beholds consistent academic records of excellence and is the recipient of several coveted scholarships, fellowships, awards & distinctions in his chosen field of research, and currently on the panel of reviewers for several national and international journals and serving active member of the National Academy of Sciences, India.

This Book

This book is an outcome of field-based research activities spanning over three decades in North-East India resulting in community-based interventional technologies for malaria vector control. It focuses on current knowledge of the mosquito vectors of human malaria, disease transmission and present-day control interventions resulting in appreciable transmission reduction. The book encompasses an illustrated account of various aspects of disease epidemiology and clearly brings out the priority areas of research for decisive attack to break the chain of malaria and poverty. Malarial threat is receding presenting a window of opportunity for scaling up interventions and developing stronger health systems for universal coverage aiming malaria elimination sooner than 2030. This book is of immediate relevance to the control programme and researchers alike reinforcing continued research on challenges ahead on road to end transmission for good towards envious goal of living in a malaria-free world.

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