Indian J Med Res 142 (Supplement), December 2015, pp 12-22
DOI:10.4103/0971-5916.176597

Malaria transmission in Tripura: disease distribution & determinants

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Received July 10, 2014

Background & objectives: **Malaria is a major public health problem in Tripura and focal disease outbreaks are of frequent occurrence. The State is co-endemic for both *Plasmodium falciparum* and *P. vivax* and transmission is perennial and persistent. The present study was aimed to review data on disease distribution to prioritize high-risk districts, and to study seasonal prevalence of disease vectors and their bionomical characteristics to help formulate vector species-specific interventions for malaria control.**

***Methods*: Data on malaria morbidity in the State were reviewed retrospectively (2008-2012) for understanding disease distribution and transmission dynamics. Cross-sectional mass blood surveys were conducted in malaria endemic villages of South Tripura district to ascertain the prevalence of malaria and proportions of parasite species. Mosquito collections were made in human dwellings of malaria endemic villages aiming at vector incrimination and to study relative abundance, resting and feeding preferences, and their present susceptibility status to DDT.**

***Results*: The study showed that malaria was widely prevalent and *P. falciparum* was the predominant infection (>90%), the remaining were *P. vivax* cases. The disease distribution, however, was uneven with large concentration of cases in districts of South Tripura and Dhalai coinciding with vast forest cover and tribal populations. Both *Anopheles minimus s.s.* and *An. baimaii* were recorded to be prevalent and observed to be highly anthropophagic and susceptible to DDT. Of these, *An. minimus* was incriminated (sporozoite infection rate 4.92%), and its bionomical characteristics revealed this species to be largely indoor resting and endophagic.**

Interpretation & conclusions: **For effective control of malaria in the State, it is recommended that diseases surveillance should be robust, and vector control interventions including DDT spray coverage, mass distribution of insecticide-treated nets/ long-lasting insecticidal nets should be intensified prioritizing population groups most at risk to avert impending disease outbreaks and spread of drug-resistant malaria.**

Key words *Anopheles baimaii* - *An. minimus* - malaria transmission - northeast India - *Plasmodium falciparum* - Tripura - vector bionomics

India is a vast country with about a billion people living at risk of malaria. Disease transmission is complex due to varied ecology, multiplicity of disease vectors and bionomical characteristics[1,2]. The northeastern region of India, which accounts for about 4 per cent of the country's population, is malaria endemic and consistently contributes 10 per cent of cases (majority of which are *Plasmodium falciparum*), and 20 per cent of reported deaths annually[3]. Among northeastern States, the State of Tripura is strategically placed for sharing a vast international border with Bangladesh (84% of its total border length) in the north, south and west. It is a landlocked State with undulating terrain and homeland to different ethnic tribes rich in diversity and cultural practices. Malaria is a major public health concern in the State with history of focal disease outbreaks characterized by high rise in cases and attributable deaths with large concentration of cases in tribal population groups living along international border [4-6]. Disease transmission is persistent and *P. falciparum* is the predominant infection (>90%); the remaining are *P. vivax* cases[7,8]. Mosquito fauna is rich and among six dominant mosquito vector species in India, both *Anopheles minimus* and *An. baimaii* are implicated as major disease vectors[9-12]. Despite decades of attempted control interventions, malaria transmission remained uninterrupted in large tracts of the land. There exists very little information on seasonal abundance and infectivity of vector mosquito species, preferred breeding sources, and their present susceptibility status to residual insecticide in use for vector control. The present study was aimed to review retrospectively, data on disease transmission for the period 2008–2012 to help prioritize high-risk districts, to understand transmission dynamics, and to study seasonal prevalence of malaria vectors and their bionomical characteristics to help formulate vector species-specific interventions for containing impending disease outbreaks.

Material & Methods

This study was conducted by the National Institute of Malaria Research (NIMR) Field Station at Guwahati, Assam, India and the study protocol was approved by the Institutional Ethics Committee. The study was based in malaria endemic blocks of South Tripura district of Tripura State. Entomological and malaria prevalence surveys were conducted during June - December 2012.

Topography, study populations and climate: The landscape of the State can be divided into the three zones, *i.e.* high physiographic zone (hills), medium physiographic zone (undulating land) and low physiographic zone (plains), and altitude varies from 12-940 meters above sea level. Tripura State is endowed with vast evergreen forest reserve (~60% of land area) rich in fauna and flora. The State is split into four districts (recently divided into eight administrative units) namely North Tripura, West Tripura, Dhalai and South Tripura. There are about 19 different tribal communities including both aborigines (*e.g*. *Tripuri*, *Reang*) and those migrated population groups (*e.g*. *Munda*, *Orang*) which together constitute about 31 per cent of the total population of the State (3.6 million)[13]. Majority of ethnic tribal population groups live largely in hilly areas each characterized by linguistic chords and rich cultural heritage. The terrain is difficult and population is sparse living in poverty along the international border areas with little awareness on disease and prevention, and have poor access to healthcare services. The area is highly receptive for malaria transmission on account of tropical climatic conditions favouring mosquito breeding, longevity and active transmission of the malaria parasite. It rains heavily on account of extended monsoons during April - September, and total annual rainfall varies from ~2-2.5 metres. Most parts of the year (March/April - September/October) are hot and humid and temperatures range from 21-34°C, with relative humidity 70-80 per cent (*Source*: India Metrological Department, Agartala).

Disease surveillance and control interventions: Under National Vector Borne Disease Control Programme (NVBDCP) guidelines, the State has a structured surveillance system in place down to the village level based on fortnightly active case detection by domiciliary visits and passive agencies supplemented by recently introduced ASHA (accredited social health activist) worker providing diagnostic and treatment services at door step[14]. Also, there exists a provision for mass blood surveys in high-risk population groups for parasite screening and treatment to contain focal disease outbreaks. For each district there is appointed District Malaria Officer. In each district, block level Primary Healthcare Centre has a diagnostic facility to ensure early case detection and treatment for visiting patients and serves as reporting centre on malaria incidence. Mosquito vector control is based on two rounds of DDT indoor residual spraying (1g/m^2) routinely conducted during March-May and then during June-August

corresponding to peak transmission periods restricted to areas reporting high incidence of malaria and death cases. Vector control interventions are strengthened by impregnation of community-owned mosquito nets with synthetic pyrethroid and/or supply of long-lasting insecticidal net (LLIN) distributed gratis among high-risk groups.

Parasitological investigations: The data based on State surveillance on malaria morbidity were reviewed retrospectively for the period 2008-2012 to ascertain disease transmission trends and identify high-risk districts for contributing most cases and deaths (*Source*: State Health Directorate of Tripura). Cross-sectional mass blood surveys were conducted in villages of Hrishyamukh (Belonia subdivision), Manubankul (Sabroom subdivision) and Silachari (Karbook subdivision) blocks of South Tripura district during June-December, 2012 to ascertain the prevalence of malaria and proportions of parasite species. In these three blocks, 28 different villages were surveyed (population, 10420) for malaria prevalence. In each study village every fourth household was reached to meet the target of about 25 per cent population coverage for blood-smear collection. A finger-prick blood sample was taken from all volunteers for screening of malaria parasite. Both thick and thin blood smears were stained with Jaswant Singh Bhattacherji (JSB) rapid stain and examined microscopically for malaria positivity and species identification, respectively. Malaria positive cases were administered radical treatment as per national drug policy in force[15]. Data for relative prevalence of malaria in different study locations were analysed by chi-square test.

Entomological survey techniques: Entomological investigations were undertaken during June-December 2012 in the same three study blocks, *i.e*. Hrishyamukh (Belonia subdivision), Manubankul (Sabroom subdivision) and Silachari (Karbook subdivision) to have representative data. To ascertain the prevalent anopheline mosquito species and their relative abundance, day-resting catches were made in 38 malaria endemic villages both in human dwellings indoor during morning hours (0600-0800h) and cattle sheds during evening hours (1800-2000h) by hand catch method aided by battery torch light. The relative abundance of mosquito species was expressed as number of mosquito adults collected per person hour. These mosquito collections were supplemented by early morning total catch by pyrethrum spray inside human dwellings and CDC miniature light traps (both indoor and outdoor) between 1900-0500 h. In addition, all night dusk-to-dawn (1900-0500 h) mosquito-landing catches were made in human dwellings (both indoor and outdoor) in villages reporting high incidence of malaria using human volunteers as bait. Mosquito adults landing on exposed body parts were collected on hourly basis to study the mosquito behaviour and identify the probable vectors for having predilection for human host. All mosquitoes thus collected were identified to the species level using regional pictorial keys[16,17]. The entomological inoculation rates (EIR) were determined as product of the human mosquito landing rate and proportions of mosquitoes carrying sporozoites in the salivary glands for estimation of transmission intensities. Among mosquito species collected, *An. minimus*, *An. baimaii* and *An. jeyporiensis* were dissected for salivary glands in 0.9 per cent saline for detection of sporozoites. These data were supplemented by host blood meal analysis using human and bovine antiserum with counter current immuno-electrophoresis technique to ascertain anthropophilic index[18]. The mosquito vector species incriminated were subject to molecular characterization based on ITS2- rDNA for correct identification of species/sibling species within the taxon as per procedures detailed by Singh *et al*[19]. To determine the species-specific breeding sites of anopheline mosquito vector species, larval collections were made from different habitats including paddy fields, seepage streams, ponds, ditches and river bed-pools. Immature stages were reared in the laboratory till emergence for confirmation of identification of the mosquito species. To ascertain present susceptibility status against diagnostic concentration of the candidate insecticide used in vector control programme, field collected mixed age female mosquito adults of *An. minimus* and *An. baimaii* were exposed to DDT (4%) using WHO's standard insecticide susceptibility test kit procedures[20].

Results

Malaria morbidity and seasonal prevalence: The data based on State disease surveillance on malaria-attributable morbidity for the years 2008-2012 are presented in Table I. During this period, total cases registered decreased from 25894 in 2008 to 11565 in 2012 inclusive of both *P. falcipraum* and *P. vivax*. Declining transmission trends were clearly discernable beginning 2011evidenced by parasite rate and annual case incidence (Fig. 1). Number of death cases recorded each year declined in parallel with reducing trends of disease transmission and every single death

Table I. Distribution of malaria cases in different districts of Tripura during 2008-2012, Northeast India

District	Year	Population	No. of blood-smears examined (% of population checked)	No. +ve for malaria (% smear positivity rate)	No. of *Plasmodium falciparum* cases (% of smear +ve cases)	Annual parasite incidence (No. of confirmed cases/ 1000 population)	Death cases
North Tripura	2008	716089	40501 (5.7)	2267 (5.6)	2078 (91.7)	3.2	11
	2009	644417	50852 (7.9)	2331 (4.6)	2242 (96.2)	3.6	39
	2010	693281	38848 (5.6)	2065 (5.3)	1996 (96.7)	3.0	4
	2011	693281	40940 (5.9)	973 (2.4)	973 (100)	1.4	4
	2012	693281	39868 (5.8)	491 (1.2)	461 (93.9)	0.7	0
West Tripura	2008	1786004	140662 (7.9)	5021 (3.6)	4745 (94.5)	2.8	11
	2009	1786558	132246 (7.4)	2765 (2.1)	2506 (90.6)	1.5	6
	2010	1780393	121902 (6.8)	2184 (1.8)	1869 (85.6)	1.2	0
	2011	1724619	117009 (6.8)	1001 (0.9)	890 (88.9)	0.6	2
	2012	1724619	111019 (6.4)	1047 (0.9)	927 (88.5)	0.6	0
Dhalai	2008	388708	56680 (14.6)	7306 (12.9)	6356 (87.0)	18.8	22
	2009	398092	69446 (17.4)	7658 (11.0)	7307 (95.4)	19.2	15
	2010	398092	56784 (14.3)	6171 (10.9)	5878 (95.3)	15.5	9
	2011	377988	53232 (14.1)	4377 (8.2)	4185 (95.6)	11.6	4
	2012	398092	43620 (11.0)	2766 (6.3)	2532 (91.5)	6.9	6
South Tripura	2008	886171	103403 (11.7)	11300 (10.9)	10409 (92.1)	12.8	7
	2009	870955	109304 (12.5)	11676 (10.7)	10897 (93.3)	13.4	2
	2010	799536	113074 (14.1)	13519 (12.0)	13065 (96.6)	16.9	2
	2011	875144	76895 (8.8)	8066 (10.5)	7764 (96.3)	9.2	2
	2012	878222	73682 (8.4)	7261 (9.9)	6995 (96.3)	8.3	1
Total	2008	3776972	341246 (9.0)	25894 (7.6)	23588 (91.1)	6.9	51
	2009	3700022	361848 (9.8)	24430 (6.8)	22952 (94.0)	6.6	62
	2010	3671302	330608 (9.0)	23939 (7.2)	22808 (95.3)	6.5	15
	2011	3671032	288076 (7.8)	14417 (5.0)	13812 (95.8)	3.9	12
	2012	3694214	268189 (7.3)	11565 (4.3)	10915 (94.4)	3.1	7

Source: State Health Directorate of Tripura (unpublished data, personal communication)

case was confirmed microscopically to be due to *P. falciparum* infection. For each year, the annual blood examination rate (% of population screened for malaria parasite) cumulative for the State remained <10 per cent (the expected rate of fever prevalence in the communities). The overall smear positivity rate (SPR) decreased from 7.6 per cent in 2008 to 4.3 per cent in 2012. Similar trends were seen for annual parasite incidences that decreased from 6.9 to 3.1 confirmed cases per thousand population/year. Among the four districts of the State, South Tripura and Dhalai were adversely affected for consistently reporting highest number of cases compared to West Tripura and North Tripura. Malaria cases were recorded in all months, however, with the commencement of the rainy season in April, there was a sudden rise in cases beginning May that peaked during June-August (Table II, Fig. 2). These were also the months of recorded high rainfall. Beginning September, there was a steady decline in cases but numbers varied with record low during December-February corresponding to dry months/low rainfall/winter season. The transmission profile was quite consistent for the years reported (2008-2012) but disease transmission trends were observed to be steadily declining.

The point prevalence study in different villages of all three study locations of South Tripura district

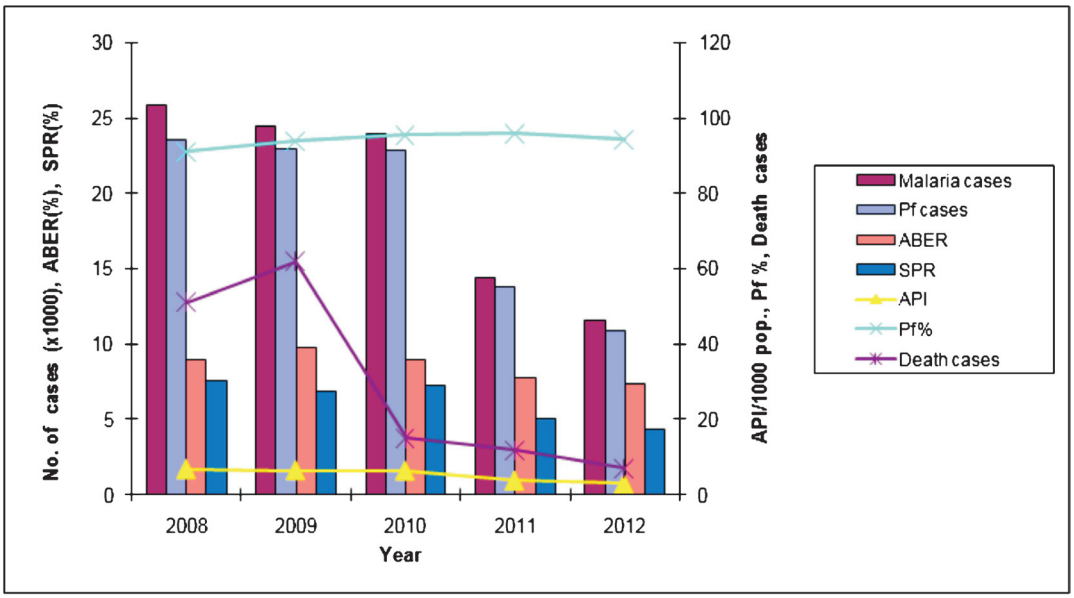

Fig. 1. Malaria-attributable morbidity and transmission trends during 2008-2012 in Tripura, Northeast India. Pf, *Plasmodium falciparum*; ABER, Annual blood-smear examination rate; SPR, % smears positive for malaria parasite; API (annual parasite incidence), number of malaria cases per thousand population (*Source*: State Health Directorate of Tripura (unpublished data, personal communication).

Table II. Meteorological data and monthly distribution of malaria cases for data based on 2012 in Tripura, Northeast India

Month 2012	Meteorological data*					No. of blood-smears examined	No. of blood-smears**		Monthly parasite incidence (no. of cases per 1000 population)	Death cases
	Rainfall (mm)	No. of rainy days	Temperature (°C) Max	Temperature (°C) Min	Average Relative Humidity (%RH)		Positive for *P. falciparum*	Positive for *P. vivax*		
January	7.2	1	24.3	11.7	79	11512	231	9	0.06	0
February	1.2	0	29.2	12.7	63	17122	280	18	0.08	0
March	2.0	0	33.4	21.6	60	20621	532	20	0.15	1
April	242.1	13	32.7	23.1	71	22575	713	41	0.20	0
May	182.4	11	34.2	24.6	73	27006	1299	99	0.38	1
June	401.0	16	32.1	26.1	82	25500	1724	93	0.49	1
July	353.7	16	32.2	25.9	81	34172	1680	153	0.50	2
August	337.0	13	32.8	25.9	81	31138	1681	79	0.48	0
September	211.4	12	32.8	25.8	83	28742	1117	64	0.32	0
October	63.7	6	32.1	23.0	80	19170	649	38	0.19	2
November	39.2	4	28.9	17.4	81	19369	587	27	0.17	0
December	1.4	0	23.6	12.3	89	11262	422	9	0.12	0

Sources: *India Meteorological Department, Meteorological Centre, Agartala, Tripura, **State Health Directorate of Tripura (unpublished data, personal communication)

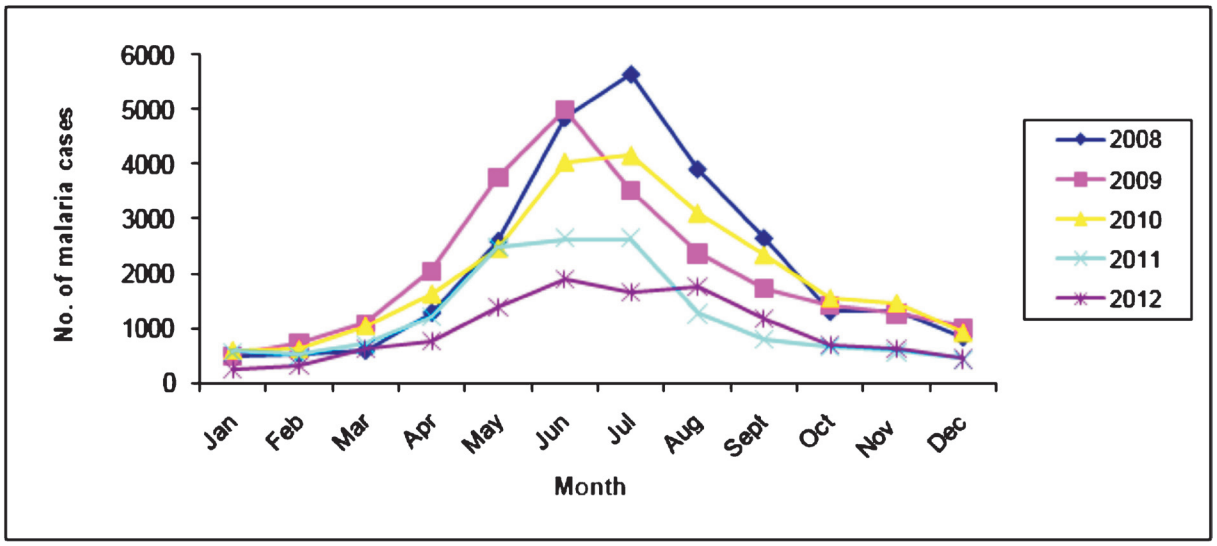

Fig. 2. Monthly distribution of malaria cases in Tripura during 2008-2012. (*Source*: State Health Directorate of Tripura (unpublished data, personal communication).

revealed that overall fever rate was 15 per cent (352/2284) but varied from 11-21 per cent between locations (Table III). Malaria cases were recorded in all age groups and differences in parasite positivity was not significant. Malaria positivity in afebrile cases ranged from 0-5 per cent and overall two per cent (38/1932) of afebrile cases were positive for malaria, majority of whom were *P. falciparum* cases (82%). In febrile cases, malaria positivity varied from 26-53 percent, and of total blood smears examined 44 per cent (156/352) were positive for malaria, of whom *P. falciparum* was the predominant infection (91%); the remaining were *P. vivax* cases. Given the heterogeneity in malaria transmission among study villages the variation in parasite prevalence between the study sites was significant (P <0.001).

Entomological observations

Mosquito abundance and resting behaviour: By various sampling techniques including day-resting catches from human dwellings indoors, evening collections from cattle sheds and CDC miniature traps (both indoor and outdoor) during wet season (June-September), 15 different anopheline mosquito species

Table III. Results of cross-sectional malaria prevalence surveys in malaria endemic blocks of South Tripura district, Tripura, Northeast India

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

Table IV. Relative abundance of anopheline mosquito species in South Tripura district, Tripura, Northeast India

Mosquito species	Day-resting (Human dwellings Indoors)		No. of mosquitoes collected in cattle biting evening collection (mosquito density per person hour) Person hours=26	No. of mosquitoes per CDC Trap (No. Trap nights)		Mean mosquito landing rate per person/night* (No. of mosquitoes collected)	
	No. of mosquitoes collected by hand catch (mosquito density per person hour) Person hours=74	No. of mosquitoes collected by total catch		Outdoor (12)	Indoor (8)	Indoor	Outdoor
Anopheles baimaii	1 (0.01)	–	1 (0.04)	0	2	3.83 (23)	3.50 (21)
An. barbirostris	–	–	66 (2.54)	21	6	–	–
An. culicifacies	–	–	1(0.04)	0	0	–	–
An. jamesii	–	–	90 (3.46)	11	2	0.16 (1)	0
An. jeyporiensis	9 (0.12)	3	20 (0.77)	18	14	2.50 (15)	3.83 (23)
An. karwari	–	–	32 (1.23)	12	0	–	–
An. kochi	–	–	64 (2.46)	36	2	–	–
An. maculatus	–	–	19 (0.73)	5	3	0.50 (3)	1.33 (8)
An. minimus	34 (0.46)	28	0	1	8	6.33 (38)	1.66 (10)
An. nigerrimus	1 (0.01)	–	72 (2.77)	47	7	0	0.16 (1)
An. nivipes/An. philippinensis	–	–	178 (6.85)	139	30	0	3.16 (19)
An. splendidus	–	–	7 (0.27)	1	0	0	0.16 (1)
An. subpictus	24 (0.32)	11	26 (1.00)	3	1	–	–
An. vagus	358 (4.84)	56	58 (2.23)	23	1	–	–
An. varuna	1 (0.01)	3	3 (0.11)	3	8	0.50 (3)	0.33 (2)

Study period: June-September, 2012, *Data based on six person nights

were identified (Table IV). Among these, *An. vagus*, *An. minimus* and *An. subpictus* were most abundant in human dwellings indoor and recorded density was 4.84, 0.46 and 0.32 per person hour respectively. These mosquito species were captured resting on walls, hanging clothes and other articles within the house typically made of split bamboo with thatched roofing. These observations were corroborated by total catch method in human dwellings for relative abundance of indoor resting mosquito populations. *An. minimus* mosquito adults collected indoors either were unfed, fully fed, semi-gravid or gravid whereas *An. baimaii* were unfed or fully fed (data not shown). In cattle biting evening catches, *An. nivipes/An. philippinensis* were most predominant; other major species included *An. jamesii, An. nigerrimus, An. barbirostris, An. kochi* and *An. vagus* in order of their relative abundance. In dry season (November-December), however, density for respective mosquito species was comparatively low in all ecotypes; *An. minimus* as well as *An. baimaii* were not recorded to occur (data not shown).

Sibling species composition of mosquito vectors: Both *An. minimus* and *An. baimaii* were subjected to molecular identification for existence of their siblings within the taxon by DNA sequencing. Of the total 44 mosquito adults of *An. minimus* and eight specimens of *An. baimaii* sequenced for ITS2- rDNA, all were characterized to be *An. minimus s.s*. and *An. baimaii* (the member species of *An. dirus* species complex), respectively, confirmed to be prevalent in Tripura.

Feeding behaviour and host preferences: *An. minimus* and *An. baimaii* mosquito species were observed to be highly anthropophilic. Of the 24 mosquito blood meals of *An. minimus,* 22 (92%), and all of *An. baimaii* (10/10) collected during July-September 2012 were positive for human blood. These observations were further substantiated by dusk-to-dawn human landing

catches in which both *An. minimus* and *An. baimaii* exhibited strong predilection for human blood meal. *An. minimus,* however, was mostly endophagic (mean mosquito landing rate 6.33 per person night), and biting activity occurred during 2100-0300 h but it was more pronounced midnight onwards till 0300 h. In contrast, *An. baimaii* searched human host equally both indoor and outdoor and mean mosquito landing rate was 3.83 and 3.50 per person, respectively, and peak biting activity occurred during 2100 h till midnight. *An. jeyporiensis* was observed active throughout the night both indoor and outdoor and mean mosquito landing rate varied from 2.50-3.83 per person. *An. nivipes/An. philippinensis* were exclusively exophagic and biting activity occurred all through the night, and mean mosquito landing rate was 3.16 per person night. *An. maculatus* was also mostly exophagic and mosquito landing rate varied from 0.50 to 1.33 per person night.

Vector incrimination: The salivary glands of mosquito adults collected from human dwellings indoors and dusk-to-dawn human landing catches were dissected for detection of sporozoites. Of the total 61 *An. minimus* mosquitoes dissected, three (4.92%) were sporozoite positive. All other mosquito species including *An. baimaii* (0/24) and *An. jeyporiensis* (0/18) were sporozoite negative.

Breeding habitats: Mosquito breeding was recorded in a variety of habitats including ponds, streams, ditches, paddy field and river beds. Of various prevalent *Anopheles* species, *An. barbirostris, An. jamesii, An. maculatus* and *An. varuna* were observed breeding in almost all habitats; however, *An. minimus* were recorded exclusively in slow flowing seepage water streams with grassy banks. Paddy fields were the most preferred habitat for most prevalent *Anopheles* species except for *An. minimus, An, karwari*, and *An. subpictus*. Instead *An. nivipes/An. philippinensis, An. splendidus* and *An. tessellatus* were recorded exclusively in paddy fields.

Insecticide susceptibility status: The field collected mixed aged mosquito adults of *An. minimus* and *An. baimaii* were exposed to DDT (4%), the currently used insecticide in vector control programme in the State. For both these mosquito species (even though number of mosquitoes exposed remained inadequate), 100 per cent mortality was observed and both *An. minimus* and *An. baimaii* were assessed to be fully susceptible (Table V).

Mosquito vectors, infectivity and relative risk: Though both *An. minimus* and *An. baimaii* were abundant and actively fed on human host, but only *An. minimus* was incriminated by detection of motile sporozoites in salivary glands; all *An. baimaii* mosquitoes dissected were sporozoite negative. However, EIRs (product of mosquito landing rate x sporozoite infection rate) for *An. minimus* varied between locations investigated (Table VI).

Discussion

The northeast region of India is the major contributor for drug-resistant varieties of *P. falciparum* malaria but the relative proportions of *P. falciparum* and *P. vivax* varies across its landscape[21]. Among the northeastern States, Tripura consistently contributed majority *P. falciparum* (>90%) cases[22]. Our study results were in conformity with the State surveillance data for *P. falciparum* being the predominant infection. *P. vivax* malaria constituted <10 per cent of cases each year and little is known of its present susceptibility status to chloroquine and relapsing pattern. Given the contextual determinants there exists every possibility of total replacement of *P. vivax* with that of drug-resistant

Table V. Insecticide susceptibility status of adult mosquito vector populations in malaria endemic villages of South Tripura district, Tripura, Northeast India

Mosquito species	Insecticide (diagnostic conc.)	No. of mosquitoes exposed (No. of replicates)	No. of mosquitoes dead post 24 h	Mortality (%)	Susceptibility status
An. minimus	DDT (4%)	40 (4)	40	40/40 (100)	S
	Control	10 (1)	0	0	_
An. baimaii	DDT (4%)	10 (1)	10	10/10 (100)	S
	Control	10 (1)	0	0	_

Study period: July-September, 2012, S, susceptible

Table VI. Mosquito landing rates, sporozoite infectivity and transmission intensities of malaria in different blocks of South Tripura district, Tripura, Northeast India

Study location	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	June-July 2012	*An. minimus*	8.25	37 (0.054)	0.44	46.4	6.1
		An. baimaii	6.00	14 (0)	0		
Manubankul	July 2012	*An. minimus*	0.50	4 (0)	0	40.7	1.3
		An. baimaii	4.00	10 (0)	0		
Silachari	September, 2012	*An. minimus*	3.25	20 (0.05)	0.16	25.0	1.3
		An. baimaii	0	0	0		

*Data based on four person nights at each location, EIR, entomological inoculation rate

malaria similar to reports in central India[23]. For treatment of *P. falciparum* malaria, there are already reports of drug resistance to artemisinin based combination therapy in Tripura[24]. The problem of malaria control continues to be complex along international borders due to poor inter-country coordinated vector control interventions, illiteracy, and large concentrations of ethnic tribes living with poor access to healthcare services resulting in hidden parasite reservoir and continued transmission[25,26].

Malaria receptivity, varied between districts evidenced by number of reported cases, a pattern that was consistent with districts of South Tripura and Dhalai reporting highest number of cases in comparison to West Tripura and North Tripura corresponding to proportional concentration of tribal populations and forest cover. The transmission was typically perennial and persistent with seasonal peak corresponding to months of heavy rainfall evidenced by monthly distribution of cases, but disease trends were clearly and steadily declining. This phenomenon could be ascribed to induction of newer interventions including artemisinin-based combination therapy for treatment of *P. falciparum* malaria and mass impregnation of community-owned mosquito nests/induction of long-lasting insecticidal nets for personal protection against mosquito bites. Similarly reducing number of malaria-attributable death cases could be due to induction of rapid diagnostic test kits for on-the-spot diagnosis and treatment of *P. falciparum* malaria. Cross-sectional mass blood surveys, however, revealed that malaria was widely prevalent both in febrile and in afebrile cases but transmission was heterogeneous.

The present study revealed that both *An. minimus* and *An. baimaii* were prevalent during monsoon season (June-September) corresponding to high transmission period. The population abundance of both these mosquito species varied across ecotypes evidenced by low prevalence of *An. minimus* in human dwellings indoors compared to mosquito landing rate per person/night. Among these, *An. minimus* were incriminated and proven efficient mosquito vectors in the State but role of *An. baimaii* could not be substantiated clearly for lack of sporozoite infectivity. Furthermore, data on mosquito blood meal analysis for *An. baimaii* remained inadequate for want of sufficient number of mosquitoes other than human bait catch to substantiate host preferences. Though the sample size remained inadequate, but both *An. minimus* and *An. baimaii* mosquito species were reaffirmed to be fully susceptible to DDT. The present data on seasonal abundance and infectivity suggested that the spray coverage for years together remained patchy and inadequate for control of vector populations. Molecular assays reaffirmed that of the *An. minimus* and *An. dirus* species complex, *An. minimus s.s*. and *An. baimaii* were the only member species prevalent in Tripura similar to reports from other northeastern States[19,27]. However, bionomical characteristics of both these species varied. *An. minimus* was largely indoor resting species (endophilic) and endophagic, and slow flowing seepage water streams were the species-specific breeding habitats. In contrast, *An. baimaii* mosquitoes were not recorded resting indoors (largely exophilic) but actively searched human host both indoors and outdoors. *An. jeyporiensis* might as well be probable vector in this region for having

indoor resting characteristics like that of *An. minimus* and biting preferences similar to *An. baimaii* calling for additional investigations.

It is hypothesized that population densities of both *An. minimus* and *An. baimaii* are rapidly depleting in the face of ecological changes and interventions in force as evidenced by disease transmission reduction. But the challenge persists and strategic reforms are needed for continuing battle against malaria[28,29]. Given the bionomical characteristics of mosquito vector species in Tripura, it is proposed that DDT spray coverage, mass distribution of insecticide-treated nets/ long-lasting insecticidal nets coupled with increased awareness for disease prevention should be intensified prioritizing high-risk districts.

Acknowledgment

The authors thank the State Programme Officer, Tripura; the Medical Officer Incharge of Silachari, Hrishyamukh and Manubankul Block Primary Health Centers; the District Malaria Officer, South Tripura, and Shrimati Swapna Sinha, Medical Officer, Udaipur for logistics support and consultations; and Dr. P.K. Mohapatra, Regional Medical research Centre, Dibrugarh, for statistical analyses. The study was financially supported by the Indian Council of Medical Research under North-East Fund assistance initiative (document number HIV/18/08/36/2010-ECD-II). Meteorological data were provided by the India Meteorological Department, Meteorological Centre, Agartala.

Conflicts of Interest: None.

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