

Research Article

Role of *Anopheles baimaii*: potential vector of epidemic outbreak in Tripura, North-east India

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Background

Malaria is one of the foremost public health concerns in north-east India. The state of Tripura, belonging to north-east India recently reported an outbreak of malaria during 2014 with thousands of positive cases and several deaths due to malaria. The epidemic has occurred mainly in the high transmission areas affecting the young population. This study was undertaken to find out the vector species responsible for the outbreak.

Methodology

House frequenting female anophelines were collected by using CDC (Centers for Disease Control and Prevention) miniature light traps. Species confirmation of *Anopheles (An.) baimaii* and *An. minimus* and presence of malaria parasite in mosquitoes was done by rDNA based molecular identification method.

Results

A rapid entomological survey revealed the involvement of *An. baimaii* on the basis of evidences like high salivary gland infectivity to *P. falciparum* (11%), dominant presence over other anophelines and exclusive anthropophily, detected during the epidemic.

Conclusion

This is the first report of incrimination of *An. baimaii* as vector of malaria in Tripura. Encroachment of dense forested areas for shifting cultivation (Jhum) and other activities, suitable environmental condition for vector propagation, and other factors like inadequate vector control and surveillance in remote areas seems to be the probable causes responsible for this epidemic.

With only 3.7% of the Indian population, North-East (NE) India has been contributing 8-12% malaria, 10-20% *Plasmodium falciparum* (*P. falciparum*) cases and about 14.78% of deaths due to malaria in the country's average.¹ North-East region of India comprises of eight states of which Tripura is considered to be the smallest with a population of 3.679 million (Census 2011). Tripura is known to be endemic for malaria with preponderance of *P. falciparum*.¹ The distribution of the disease is not uniform in Tripura and shows wide variation both spatially as well as temporarily. However, frequent outbreaks of malaria in different states of NE region prior to 2008 used to be very common.²⁻⁵ In recent times, the outbreak of malaria has been declining due to large scale introduction of artemisinin based combination therapy as well as wide spread use of insecticide treated bed nets (ITBN) and LLIN (Long lasting insecticide treated bed nets) distribution along with indoor residual spray (IRS). However, an exception occurred in 2014 in the form of a severe malaria outbreak in Tripura leading to high morbidity and mortality. Historically, the outbreak of

malaria in NE India is attributed by *Anopheles minimus* (*An. minimus*) and usually limited to or start from foot hill area. Till date, *An. baimaii* is found to maintain high endemicity of malaria in stable malaria condition. In tribal areas of NE states malaria epidemics are rare. As the information on role of the malaria vectors in Tripura are limited and earlier studies reported the presence of *An. minimus* confirming its role in malaria transmission in Tripura. We carried out an entomological investigation to find out the role of different vectors of malaria in the epidemic of Tripura and found *An. baimaii* to be the vector responsible for this epidemic.

METHODS

STUDY AREA

Tripura is a small north-eastern state of India, situated between latitude 22°57' N and 24° 33' N and longitude 91° 10' and 92°20' E, having a 865 Km international border with Bangladesh from three sides and with a narrow neck border-

ing 109 KM with Mizoram and 53 Km with the state of Assam. With an annual rainfall of above 2000 mm, the climate of this state is moist and humid with temperature ranging from 21° C to 38° C in summer. Topographically, 60% of the geographical area of the state is hilly and covered by forest. Entomological surveys were made in three districts of Tripura namely West Tripura, Khowai and Dhalai districts. Among these three districts Dhalai was the most affected in the reported epidemic followed by Khowai and West Tripura respectively. Mosquitoes were collected from two villages of West Tripura, two villages of Khowai and one village of Dhalai districts. These villages are having similar ecological settings and situated in hilly forested terrain.

ENTOMOLOGICAL INVESTIGATION

House frequenting female anophelines were collected by using CDC (Centers for Disease Control and Prevention) miniature light traps. Traps were placed at a height of about 1.5 to 2 m from the floor adjacent to the beds where the residents usually sleep. Traps were operated from dusk to dawn and in the morning trapped mosquitoes were brought to the field laboratory. In the laboratory mosquitoes were identified to species or species complex level by using standard keys⁵ and kept individually after assigning a unique identification number in beam capsules containing silica gel.

Species confirmation of the morphologically identified *An. dirus* and *An. minimus* complex mosquitoes, presence of human blood in the mosquito abdomen and detection of *Plasmodium* species in the gland and gut of the collected anophelines were carried out by using molecular method. The head-thorax and abdomen portion of individual mosquitoes were bisected individually and homogenized in 1.5ml eppendorf tubes separately. To the homogenate 60µl of Phosphate Buffer Saline (PBS) was added and mixed well. The lysate was then pipetted out in FTA classic cards and allowed to dry overnight in laminar flow. The DNA was isolated from these FTA cards according to manufacturer's instruction (Whatman, Kent; UK).

For the species confirmation of the Dirus complex and Minimus complex mosquitoes, the allele specific polymerase chain reaction (ASPCR) assay based on rDNA developed by Walton et al⁶ and Phuck et al⁷ was used with minor modifications. Presence of *Plasmodium* infection in individual mosquitoes were detected both in head/thorax and abdomen separately for the detection of gland and gut infection by using the nested polymerase chain reaction method developed by Snounou et al.⁸ This rDNA based method uses genus specific primers for the first round of amplification and in the second round each parasite species is detected separately by using species specific primers. Human blood in the abdomen of blood fed specimens of Dirus and Minimus complex of mosquitoes was detected by following the protocol and primers as described by Mohanty et al.⁹

RESULTS

ANOPHELINE DIVERSITY

A total of 124 anopheles mosquitoes belonging to 11 species were found during the survey. Total number of *An.*

baimaii identified during the survey was 63 (about 50%). In comparison to *An. baimaii* the prevalence of the other vector species from north east India was found to be less, as only 8% of the total collection was composed *An. minimus* (Table 1). Species identification of *An. baimaii* and *An. minimus* was confirmed by using rDNA based molecular species identification method.

AVERAGE TRAP DENSITY, PLASMODIUM INFECTIONS & HUMAN BLOOD INDEX

In the present study, 11% (n=7) of the total *An. baimaii* (n=63) were found to harbor *Plasmodium falciparum* (Table 2). *An. baimaii* was found to be present in all five villages during the epidemic. Mosquito per trap density was recorded to be lowest in a village of Jirania primary health centre (PHC), West Tripura, unaffected by the reported malaria epidemic, whereas, peak density of *An. baimaii* was noted in a highly affected village of Mungiakamin PHC (Table 3) under Dhalai District. Human blood index of *An. baimaii* was recorded to be 100% in 22 nos. of blood fed specimens screened.

DISCUSSION

The earliest and only record of vector incrimination relating to human malaria in Tripura state was in 1954, when *An. minimus* was incriminated for malaria sporozoites.¹⁰ However, the role of the other important vector *An. baimaii* and of various *Anopheles* species present in Tripura state could not be substantiated clearly. *An. minimus* and *An. baimaii* (earlier *An. dirus* D) are the two primary vectors of human malaria in the north-eastern region of India.⁵ Earlier studies in Tripura confirmed the presence of only *An. baimaii* among the members of Dirus complex by using molecular methods in specimens collected from Dhalai, North Tripura, South Tripura and West Tripura districts.¹¹

Forest Survey of India (2011)¹² using satellite data of 2009 stated that 76% of the geographical area of Tripura state is under forest. About 109 km² area under very dense forest, 4,686 km² area under moderately dense forest and 3182 km² under open forest. In the presence of highly efficient vector like *An. baimaii*, adapted to hilly forested habitats and *An. minimus* adapted to foothills, forest fringes and deforested hills, the malaria epidemiology of north eastern region of India is highly complex. Situation is more difficult due to the presence of several secondary vectors, whose roles are yet to be defined. The relationship of malaria transmission with forest cover and deforestation is a complex, regionally distinctive and locally specific phenomena.¹³ Closed or deep forested areas are the natural habitat of forest adapted hygrophilic and exophilic species like *An. baimaii* which can spread over to the edge of the forest and fragmented forest areas during the rainy season due to the increase in humidity and breeding habitats. Human settlers within and in the vicinity of forest was considered to be a probable cause for increasing the densities of highly anthropophilic forest species like *An. baimaii* by providing hosts and transitory pools for egg laying.¹⁴ In contrast *An. minimus*, the other primary vector species of this region, adapts to near forest areas like foothills, forest fringes and

Table 1. Anopheles mosquito species collected from five villages in Tripura

Sl. No.	Species name	Nos. collected	% composition
1	<i>An. baimaii</i>	63	50%
2	<i>An. jeyporiensis</i>	14	11%
3	<i>An. minimus</i>	10	8%
4	<i>An. varuna</i>	10	8%
5	<i>An. kochi</i>	8	6%
6	<i>An. barbirostris</i>	5	4%
7	<i>An. phili/nivipes</i>	5	4%
8	<i>An. maculates group</i>	4	3%
9	<i>An. vagus</i>	3	2%
10	<i>An. hyrcanus group</i>	1	0.8%
11	<i>An. aconitus</i>	1	0.8%

Table 2. Average trap density, Plasmodium infections & human blood index in collected mosquito species

Sl. No.	Species	Average per trap per night density		Plasmodium infections	
		Total collected (% composition)	Per trap night	Gland	Gut
1	<i>An. baimaii</i>	63	4	7 (n=63)	2 (n=56)
2	<i>An. minimus</i>	10	0.62	0 (n=10)	0 (n=10)
3	<i>An. jeyporiensis</i>	14	0.87	0 (n=14)	0 (n=14)
4	<i>An. varuna</i>	10	0.62	0 (n=10)	0 (n=10)
5	<i>An. kochi</i>	8	0.5	0 (n=8)	0 (n=8)
6	<i>An. barbirostris</i>	5	0.31	0 (n=5)	0 (n=5)
7	<i>An. phili/nivipes</i>	5	0.31	0 (n=5)	0 (n=5)
8	<i>An. maculates group</i>	4	0.25	0 (n=4)	0 (n=4)
9	<i>An. vagus</i>	3	0.18	0 (n=3)	0 (n=3)
10	<i>An. hyrcanus group</i>	1	0.06	0 (n=1)	0 (n=1)
11	<i>An. aconitus</i>	1	0.06	0 (n=1)	0 (n=1)

deforested hilly areas. However, deforestation followed by tree crop plantation may again facilitate *An. baimaii*. *An. baimaii* a member of the Dirus complex adapts so well to rubber plantation that it was presumed to be even better than to their original tropical rain forest habitats.^{15–18} *An. baimaii* breeds in the shaded transient pools of a forest with good canopy cover. However, practice of jhum cultivation opens up the forest and creates favorable breeding habitat for *An. minimus* in the associated streams as absence of shade promotes growth of vegetation along the edges of the streams.¹⁹ Considering the scenario Guerra et al¹³ remarked that malaria situation in such areas are highly complex, unpredictable and not unidirectional. In the backdrop of climate change and its effects on the malaria situation may be more complex in the coming days.

For the propagation of *An. baimaii*, the pattern of rainfall seems to be more important as their density showed a significant positive correlation with rainfall two weeks before.¹¹ Heavy rains can flush out the larval habitats and can also dilute the nutrients for the larvae.¹⁴ Intermittent rains not to the extent of flushing out the breeding habitats of *An. baimaii* and a 3 to 5°C higher mean temperature during the period has shortened the life cycle of the vector and reduced extrinsic cycle of the malaria parasite might have cre-

ated ideal condition for vector expansion and malaria transmission. Meteorological data for Tripura from 1st quarter of April to 4th quarter of June, just before the onset of the epidemic, also depicted an intermittent and deficient rainfall pattern during this period in comparison to previous year. Jhum cultivation is widely practiced by the indigenous people of Tripura and a sizeable population in the hills depends on Jhum for their livelihood.²⁰ Dhalai district which has a considerable population of Jhumias (people involved in shifting cultivation) was also recorded to be the most worst affected district in the reported epidemic. Due to population growth and for livelihood there is not only a reduction of jhum cycle from 10/20 years to 2/3 years but also more and more peoples are encroaching virgin dense forested areas for shifting cultivation and other activities leading to creation of high malaria endemic areas having perennial transmission near the villages of Ganganagar and Chowmanu PHC of Dhalai district.

In the present study *An. baimaii* was found to be the dominant species constituting 50% of the total collection (Table 3). This is much higher compared to our earlier survey where this species was found in fewer numbers Prakash et al.¹¹ This indicates an expansion of this species during the present time under favorable condition.

Table 3. Entomological collections using CDC light traps in malaria affected villages of Tripura

SPECIES NAME	WEST TRIPURA				KHOWAI				DHALAI
	MANDAI PHC		JIRONIA PHC		TRILOSIKHAR PHC		MUNGIKAMIN PHC		GANGANAGAR PHC
	HARBUNG VILLAGE		SINAI VILLAGE		UDAN MAIDAN VILLAGE		HUMBAI VILLAGE		
	NO.	PER TRAP	NO.	PER TRAP	NO.	PER TRAP	NO.	PER TRAP	NO
<i>An. aconitus</i>	-	-	1	0.25	-	-	-	-	-
<i>An. barbirostris</i>	1	0.3	1	0.25	1	0.3	3	1	-
<i>An. baimaii</i>	12	4	2	0.5	5	1.7	37	9.2	7
<i>An. hyrcanus</i> group	-	-	1	0.25	-	-	-	-	-
<i>An. jeyporiensis</i>	-	-	7	1.8	-	-	8	2.7	-
<i>An. kochi</i>	-	-	3	0.8	-	-	5	1.7	-
<i>An. minimus</i>	-	-	-	-	9	3	-	-	1
<i>An. maculatus</i> group	2	0.7	1	0.25	-	-	-	-	-
<i>An. nivipes</i>	-	-	5	1.2	-	-	1	0.3	-
<i>An. varuna</i>	-	-	-	-	-	-	-	-	1
<i>An. vagus</i>	-	-	3	0.8	-	-	1	0.3	-

PHC – primary health centre

Our study revealed 11% *An. baimaii* (n=63) infected with *Plasmodium falciparum* by using molecular assay indicating that the species is playing a major role in the present epidemic (Table 2). Sporozoite rates of 14% were reported in *An. dirus* s.l. from forested areas of Myanmar.²¹ In a highly anthropophilic species like *An. baimaii*, a small population of the vector can maintain a high level of transmission.¹⁵ The recorded 100% human blood index in *An. baimaii* collected during the epidemic period is in agreement with its pivotal role in the epidemic.

The importance of *An. baimaii* as a vector of human malaria in north-eastern India was first noted when malaria persisted even after achieving excellent control of *An. minimus* by using DDT as indoor residual spray.¹⁷ Exclusively exophilic nature of this species makes it difficult to control by using Indoor residual spray.¹⁴ DDT spray coverage was found to be poor in Tripura state. Spray virtually started from the month of June in Tripura in the epidemic year and hence before its completion the epidemic already appeared. Although DDT might be comparatively less effective for this species, still the spray could have reduced the man-vector contact to some extent and is effective against the other important vector *An. minimus* from NE India. A study from Bangladesh shown that *An. baimaii* avoid sprayed huts by biting more in outdoors.²² In Thailand DDT treated huts showed a decline of 79.4% in attempted blood feeding by *An. dirus* s.l.²³ Looking into the biting habit of *An. baimaii* in north-eastern India a sizeable malaria transmission risk exists during the pre-bed time (18.00-21.00 hours)²⁴ that can be avoided by using insecticide treated nets supplemented with repellent creams as reported by Prakash et al.² Whereas in case of *An. minimus* effective control can be possible by using ITNs alone as only a small proportion of this species bites before 21:00 hours.²⁵ Long lasting insecti-

dal nets (LLINs) were used in Tripura distributed by Public Health Department from 2009-12. However, coverage was not full and they were distributed without studying community acceptance of using bed nets, bed net size, sleeping pattern etc. On the basis of these findings, considering the hilly forested terrain and remoteness of many villages inhabited by these vectors in Tripura and for the difficulty of implementing vector control measures in time, scaling up of LLINs seems to be highly essential.

Behavior of *An. baimaii* like avoiding contact with the sprayed walls of the human dwellings and its early biting habit is a major constraint for achieving complete vector control even after using IRS and ITN/LLIN in combination. In this context, the situation in Tripura seems further worsened due to the inadequate implementation of these routine vector control measures.

As evidenced by our study involvement of *An. baimaii* in Tripura epidemic does not augur well for future if appropriate control measures are not undertaken as it is an exclusively anthropophilic species found in the forested areas of north east India.

CONCLUSIONS

To summarize the present situation, we can infer that human settlements in deep forested areas combined with favorable environmental condition and inadequate vector control help in the expansion of densities of forest adapted species like *An. baimaii* leading to an epidemic situation. Considering the rapid topographical changes in Tripura due to deforestation and commercial plantation coupled with the effect of climatic changes, the malaria situation in Tripura will be unpredictable in coming years.

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COMPETING INTERESTS

The authors completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available upon request from the corresponding author), and declare no conflicts of interest.

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