Parasitological, Entomological and Epidemiological studies in a high malaria-endemic area of Tripura



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Collaboration with: National Centre for Biomedical Genomics, Kalyani
North Eastern Space Application Centre, Shillong
National & Malaria Program - Tripura, Meghalaya, Mizoram, Arunachal Pradesh, West Bengal
MESA-ICEMR
Jawaharlal Nehru University, Delhi
WHO

NDMC Workshop , IIT Bombay, 29.11.2023

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- **Malaria Evolution in South Asia (South Asia-ICEMR)**

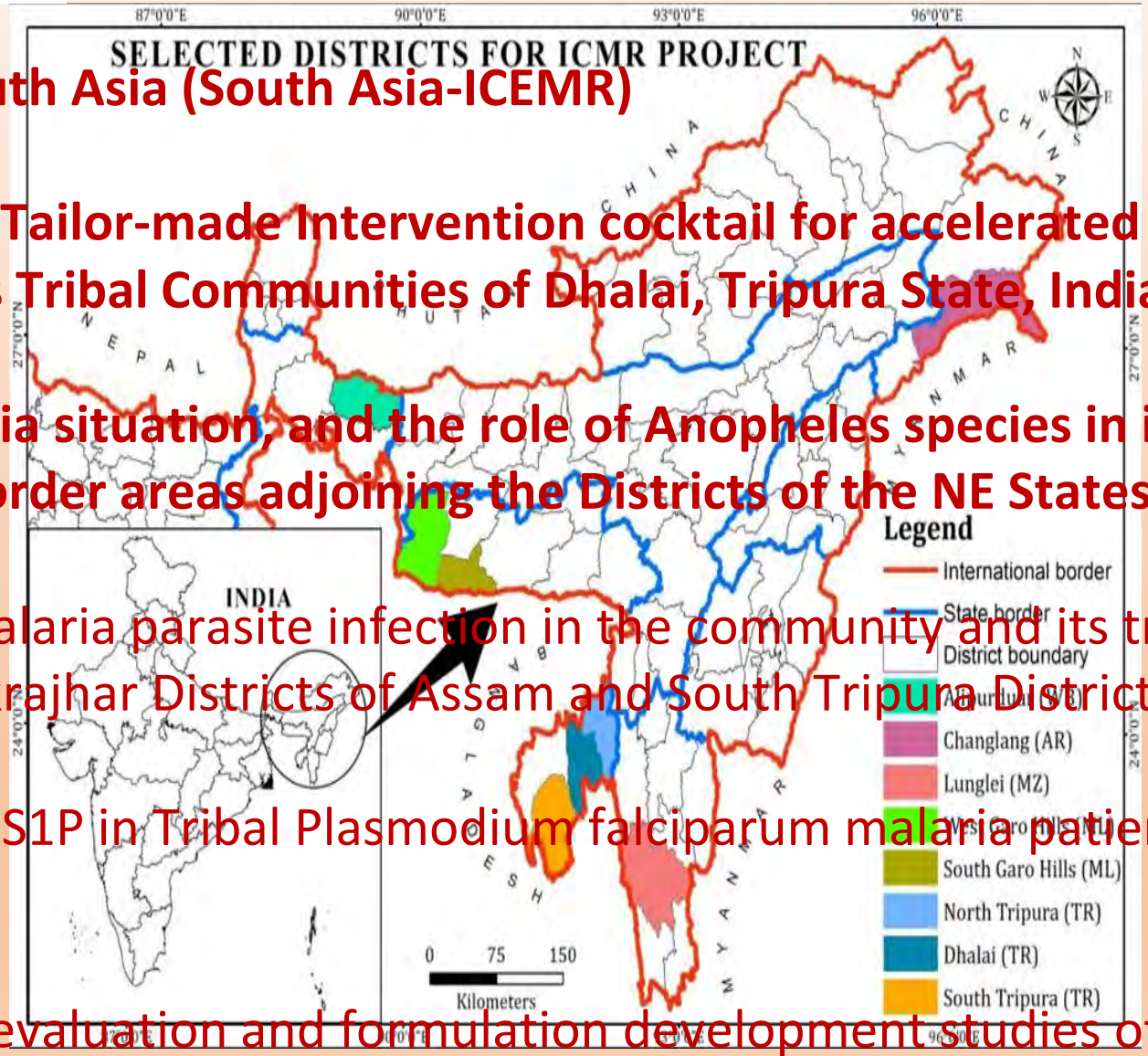
- **A Study of Targeted and Tailor-made Intervention cocktail for accelerated malaria control in Jhum (shifting) cultivators Tribal Communities of Dhalai, Tripura State, India**

- **Assessment of the malaria situation, and the role of Anopheles species in its transmission in selected International border areas adjoining the Districts of the NE States & NE corridor**

- A study of low-density malaria parasite infection in the community and its transmission potential in Udalguri, Kokrajhar Districts of Assam and South Tripura District of Tripura

- Evaluation of the role of S1P in Tribal Plasmodium falciparum malaria patients for its potential therapeutic consideration

- Antipyretic, antimalarial evaluation and formulation development studies of dried fish extracts and fermented fish preparation belonging to the Puntius Species



Studies in North East India

Recent publications

1. Bhowmick, I.P., Chutia, D., Chouhan, A., Nishant, N., Raju, P.L.N., Narain, K., Kaur, H., Pebam, R., Debnath, J., Tripura, R. and Gogoi, K., 2021. Validation of a mobile health

technology platform (Fever Tracker) for malaria surveillance in India: development and usability study. *JMIR Formative Research*, *5*(11), p.e28951.

2. Bhowmick, I.P., Nirmolia, T., Pandey, A., Subbarao, S.K., Nath, A., Senapati, S., Tripathy, D., Pebam, R., Nag, S., Roy, R. and Dasgupta, D., 2021. Dry post wintertime mass surveillance unearths a huge burden of P. vivax, and mixed infection with P. vivax P. falciparum, a threat to malaria elimination, in Dhalai, Tripura, India. *Pathogens*, *10*(10), p.1259.

3. Bhowmick, I.P., Pandey, A., Subbarao, S.K., Pebam, R., Majumder, T., Nath, A., Nandi, D., Basu, A., Sarkar, A., Majumder, S. and Debbarma, J., 2022. Diagnosis of indigenous non-malarial vector-borne infections from malaria negative samples from community and rural hospital surveillance in Dhalai District, Tripura, North-East India. *Diagnostics*, *12*(2), p.362.

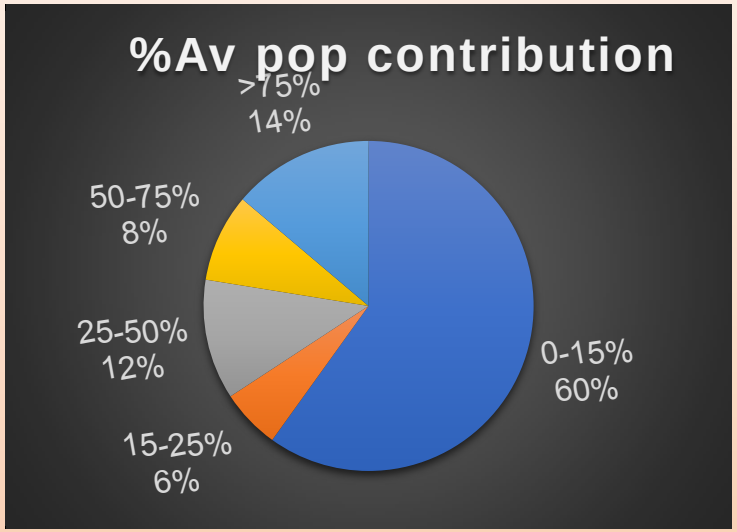
4. Biswas, S., Rajkonwar, J., Nirmolia, T., Jena, S.R., Sarkar, U., Bhattacharyya, D.R., Borkakoty, B., Pandey, A., Subbarao, S.K., Majumder, T. and Pebam, R., 2023. First report of rubber collection bowls & plastic and bamboo water containers as the major breeding source of Ae. albopictus with the indigenous transmission of Dengue and Chikungunya in rural forested malaria-endemic villages of Dhalai District, Tripura, India: The importance of molecular identification. *Biomedicines*, *11*(8), p.2186.

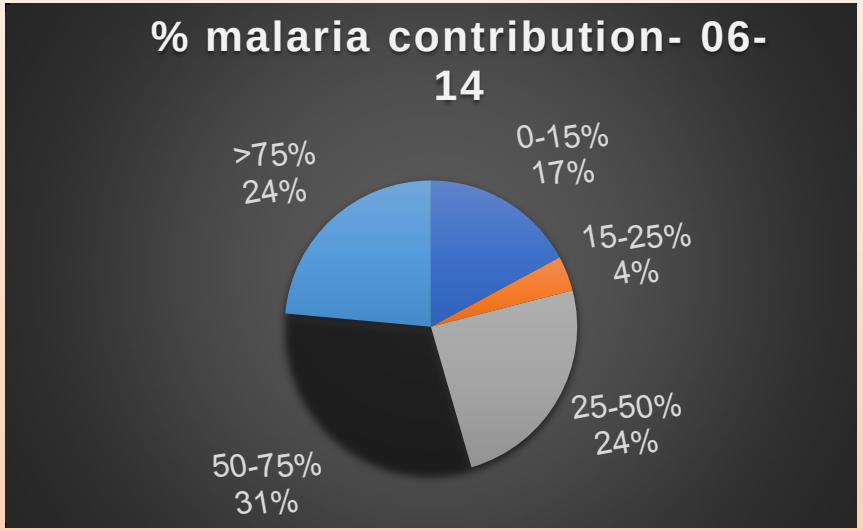
Disproportionate Malaria Burden with High *Heterogeneity* in NE India

Malaria disproportionately higher in NE India

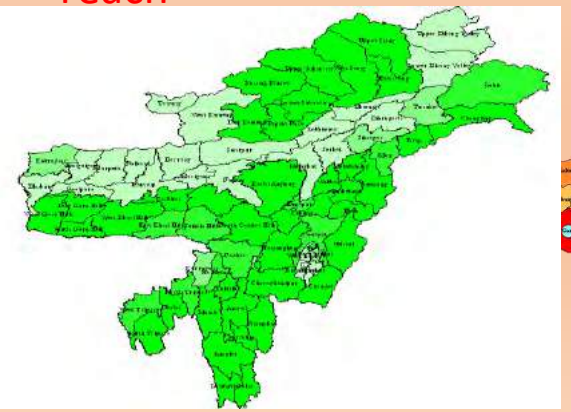
NE India contribution to India	
land cover :	8%
Population :	~3.5% ('11 census)
malaria deaths :	10-25% ('16-20)
malaria cases :	6-8% ('16-20)
P. falciparum cases	11-18% ('16-20)
Tribal Population :	~12% (2011 census)

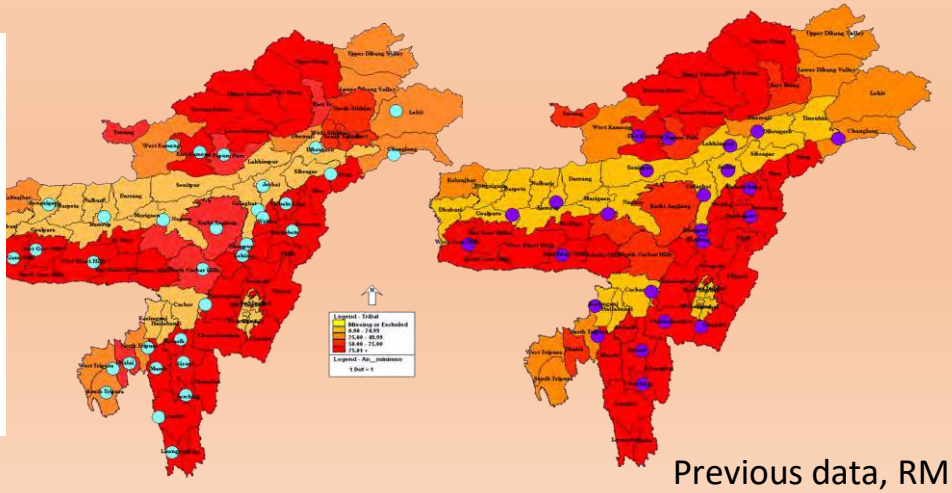
NE Malaria disproportionately higher in Tribal Population





Malaria affected Tribal Districts – **Forested**, Hard-to-reach





Previous data, RMRC

□Area Specific Gaps - Hard-to-reach ? Other factors ?

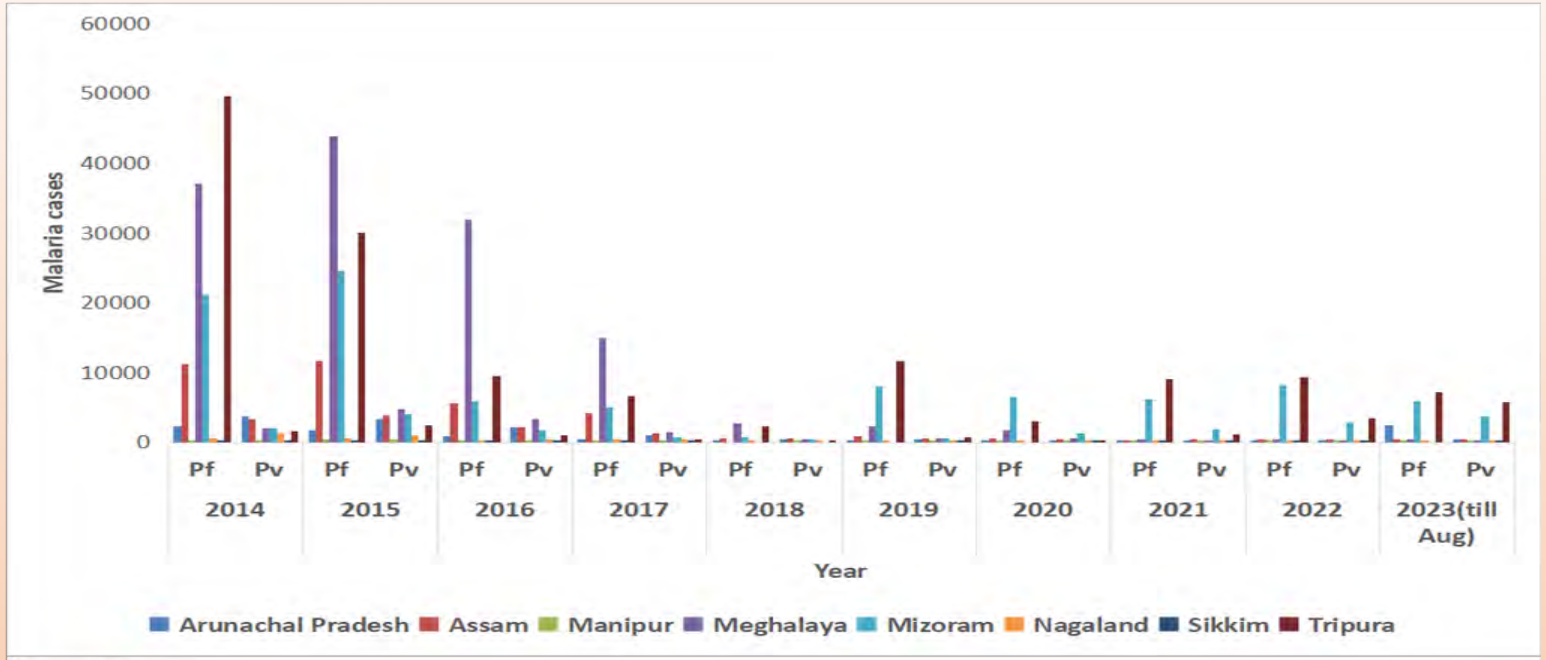
□High forest coverage and prolonged high rainfall-Unique vectors present in NE

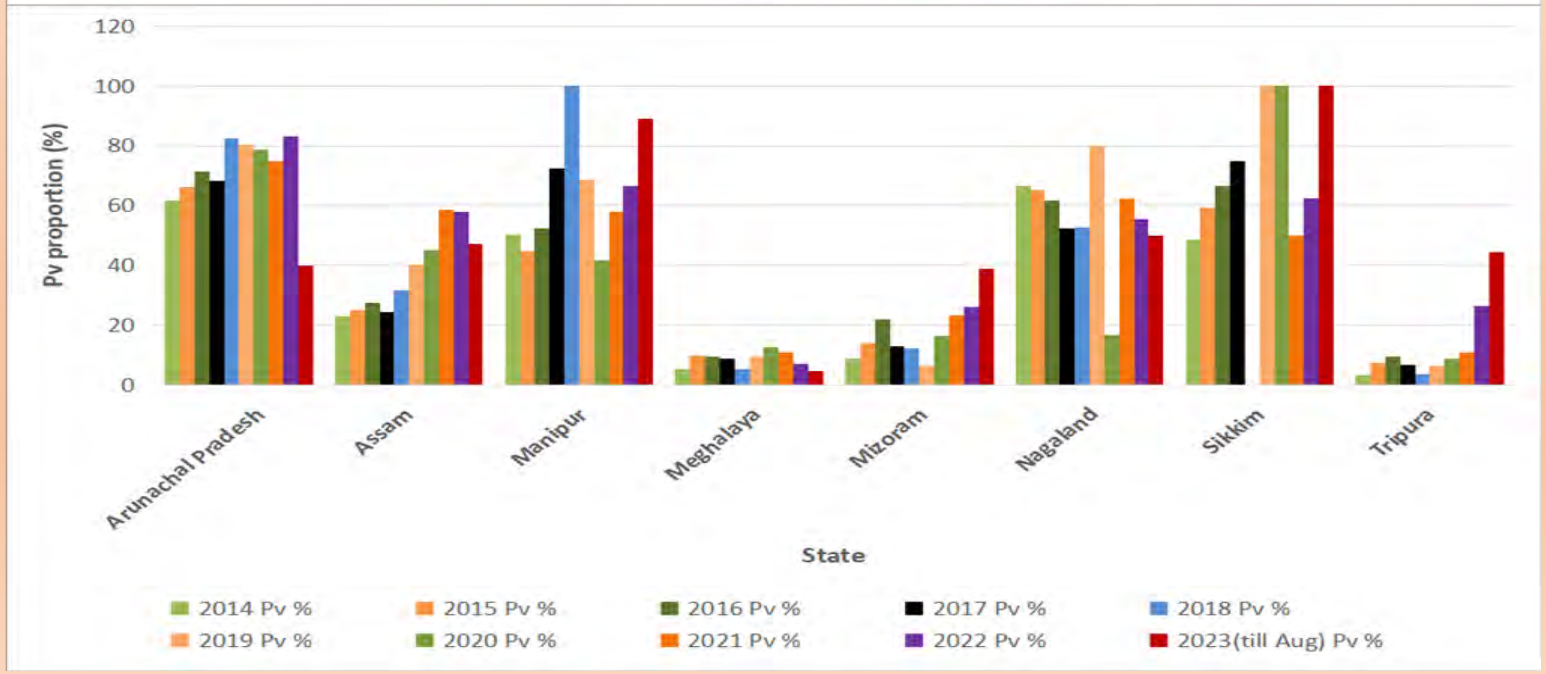
□Forest vector *An baimaii* & forest fringe vector *An minimus* found as major vectors

□Any specific Tribal Communities ?

□Hard-to-reach ? Other factors ?

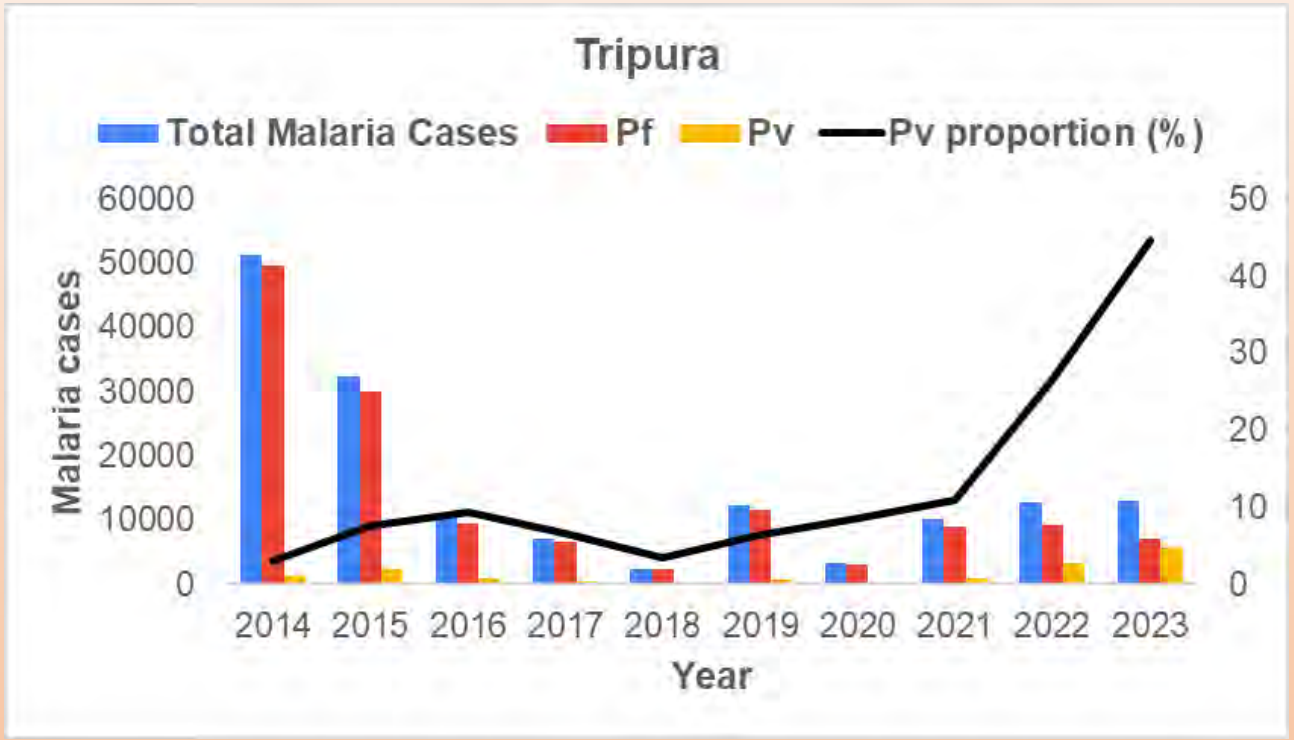
North East Case and PV% comparison from 2014 to 2023 (Aug) trends

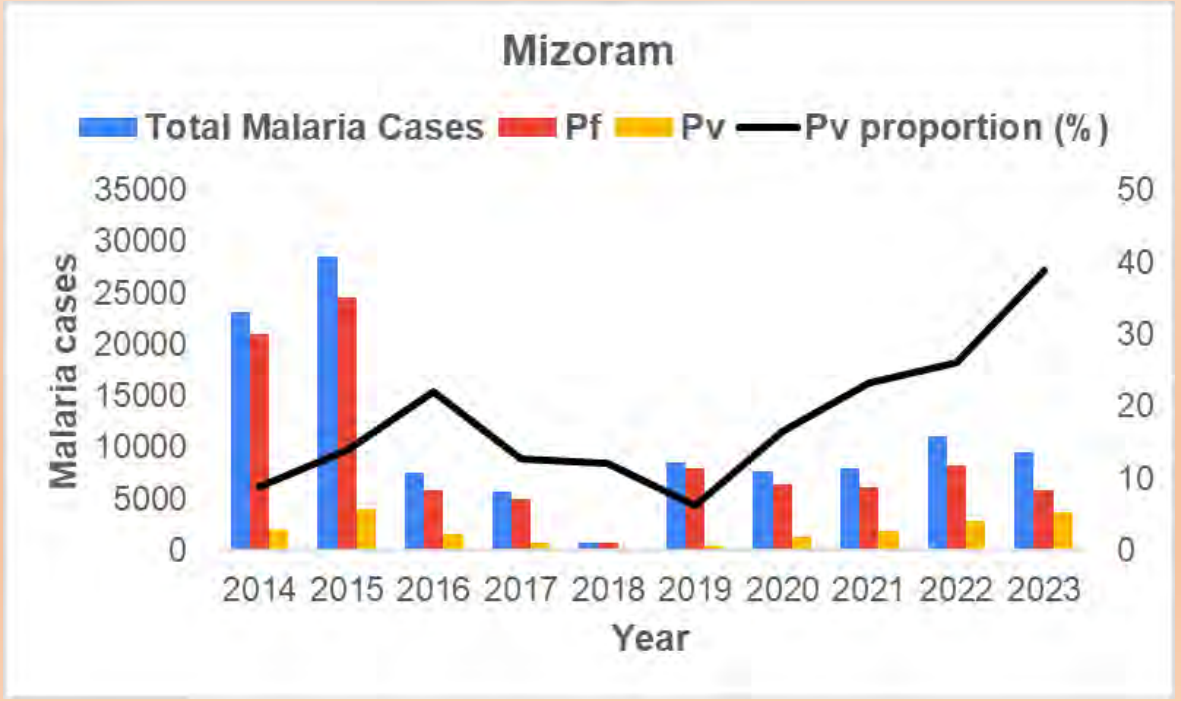




Overall trend of decreasing cases of Malaria, Pf, Pv cases in 2014-23 in India

BUT Increasing Trends of Malaria Cases in some NE States like Tripura and Mizoram with Increasing Pv

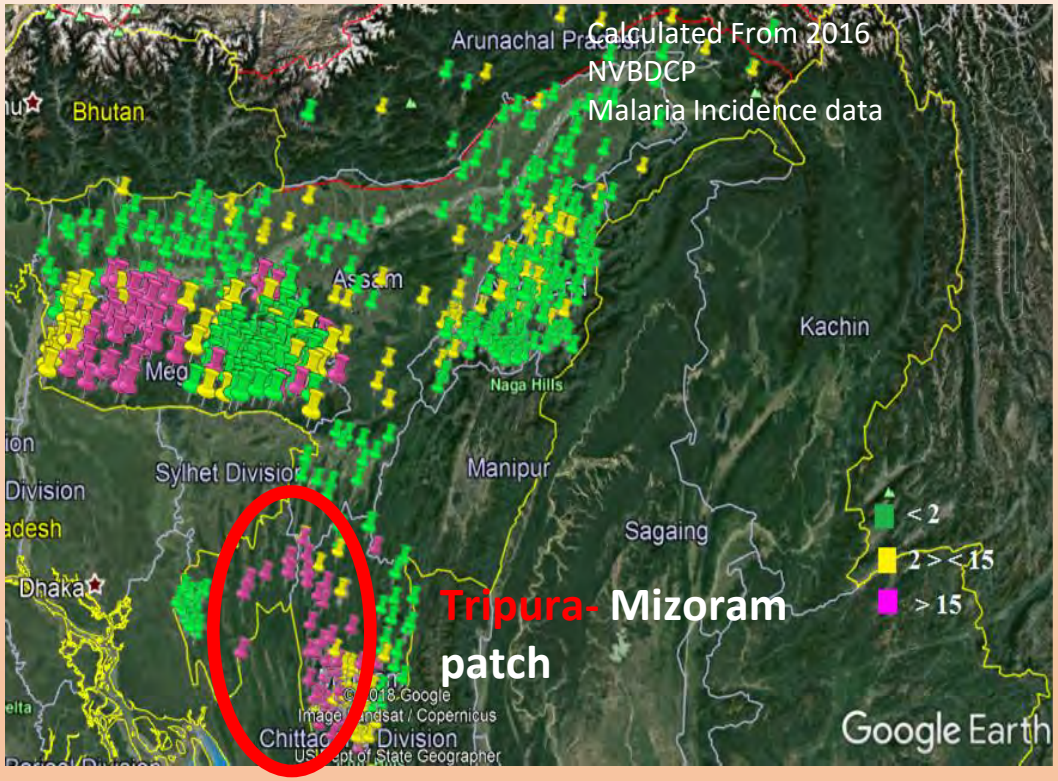




Reference : NCVBDC Data

Micro-Heterogeneity AT PHC , SC, Hamlet Level

Clustering at few Tribal Forested area PHCs



Tripura as Case Study

- **2014 Malaria Epidemic** in Tripura
  > 50,000 cases , ~100 deaths in 2 Months
- Mainly 3 Districts, 9 PHCs – Major Contributors
- ~3% of Tripura pop ~32% cases
- ~28% pop of Dhalai Dist. in 5 PHCs ~72% cases
- Clustering observed at Subcentre and Village levels

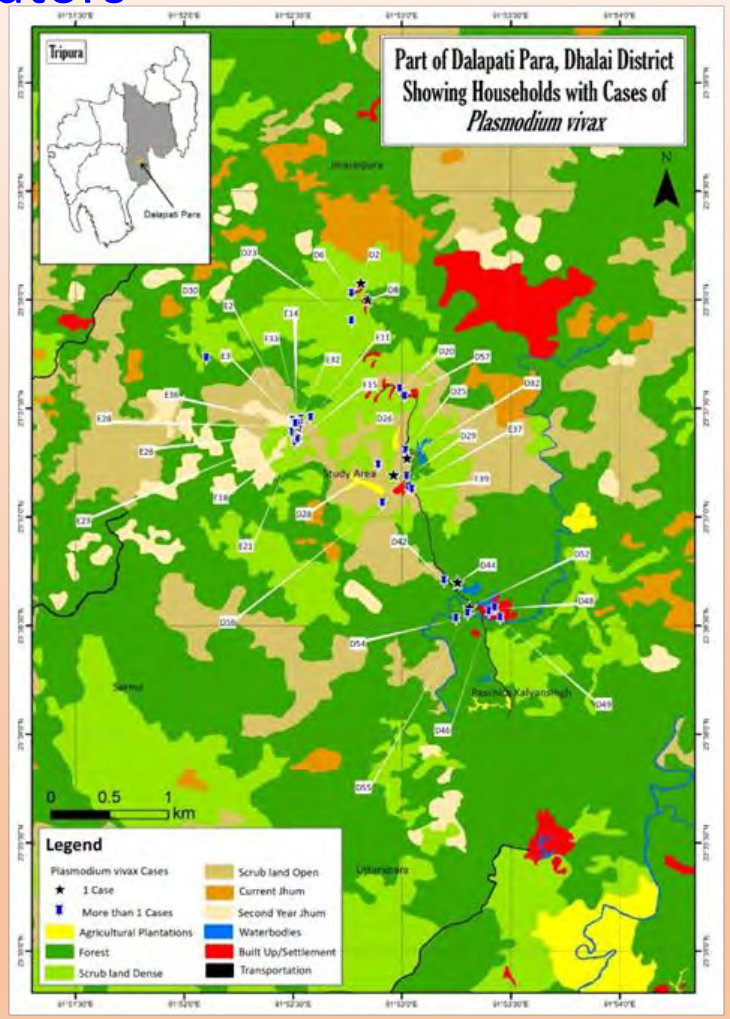
- **~30% Tribal population of Tripura , contribute >99 % of malaria**

- Epidemic affected the children in persistent foci , previously endemic areas, still persisting and increasing cases – Hot foci

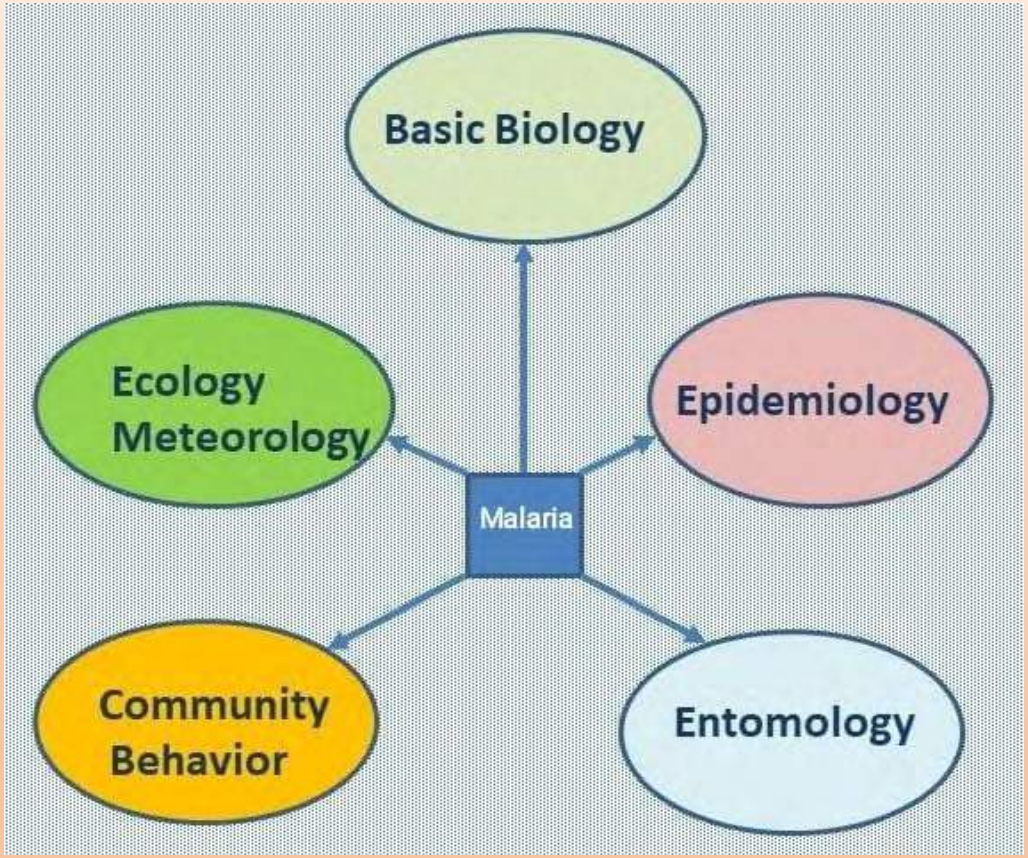
Community Survey in the highly affected villages in December 2014 (as piloting for MESA Community Survey) - majority as Jhum Cultivators

Source of Primary Income

Jhum Cultivation	359 (88.21%)
Forest worker	3 (0.74%)
Agriculture, livestock, fisheries	37 (9.09%)
Others	8 (1.97%)



MS Under Preparation



Collaborations :

- MESA-ICEMR
- North East Space Application Centre, Shillong
- State Health & Family Welfare Depts
- National Centre for Vector Borne Disease Program
- Agartala Govt Medical College
  - Depts of Community Medicine, Microbiology
- MRHRU, MRU, VRDL Tripura, AGMC
- JNU
- WHO

Jhum

Burnt forests

Probable Risk Factors

- Most of the tribal people in these areas are engaged in **Jhum (Shifting/ Slash-Burn Cultivation in Hard-to-Reach remote forested areas**

- Malaria endemic villages surrounded by forest

- Long stay in Jhum – missed by health system- **Hard-to-reach**

- **Other factors along with Hard-to-reach?**

Remote area Village hut surrounded by forest and streams

Very remote Jhum huts in hilltop forest

Different types of Jhumias:

Permanent Jhumia : Whole period Day & Night Stay

Partially permanent Jhumia: Night Stay for few months, rest of time up and down through forest

Completely Temporary Jhumia: Always up and down through forest

Effect of Land Use Land Cover and climatic variables of malaria incidence in Tripura

In Collaboration with Dr Analabha Basu, Dr Diptarup Neogi, Kalyani NIBMG

Objective/Research Question

Are there any specific Land Use Land Cover (LULC) type and/or climatic variables that influences Malaria cases/API in 2019?

Data of 2019: for 78 blocks across all the districts of Tripura.

Predictors

1. **LULC**
   a. Forest
   b. Croplands
   c. Shrublands
   d. Rubber Plantations
   e. Urban Settlements
   f. Jhum

2. Climatic Variables

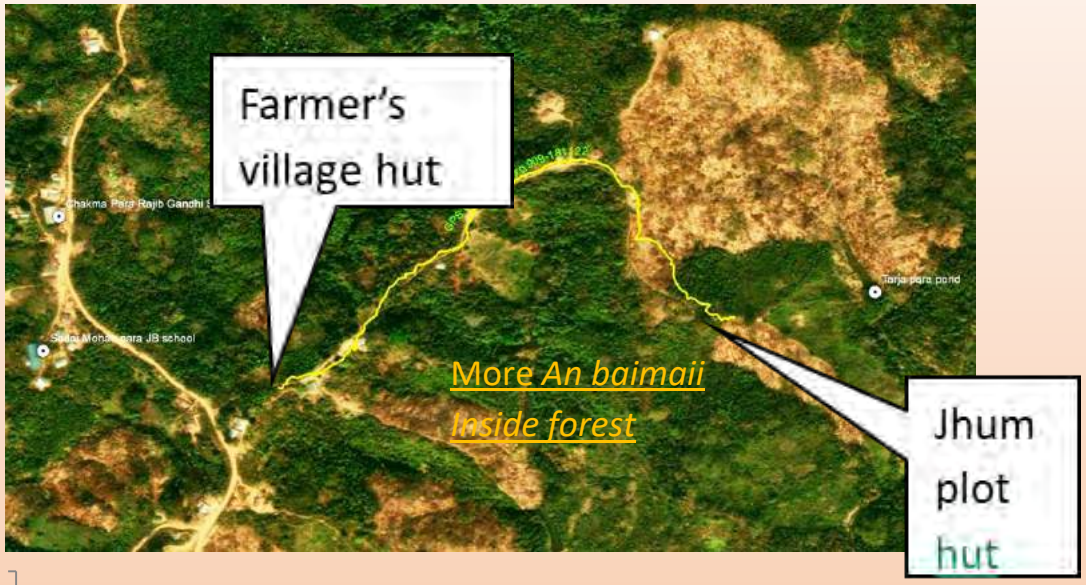
a. Temperature (Min, Max, Avg)
b. Elevation (min, max, avg)
c. Precipitation

- There were total of 13 predictor variables.
- Population size was the another variable for malaria case count.

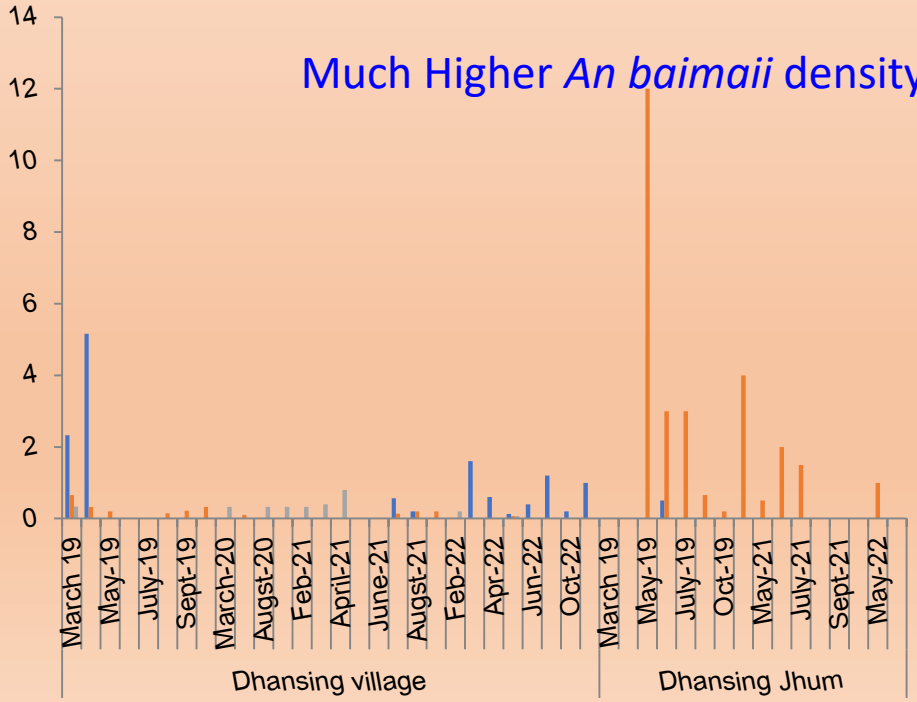
Response: Annual malaria cases (Annual Parasitic Incidence)

Establishing Jhum as Malaria Risk Factors by entomological, behavioral and epidemiological studies

A geo logger track line showing the route to jhum field from farmer's house on google earth background in Tarja Para, Tripura.



Much Higher *An baimaii* density in Jhum



Risk factors and inadequacy of present vector control measures

- ✔ Village Proximity to Forest
- Jhumias are exposing themselves during Jhum stay, Jhum to Village movement through forest other than village stay
- Chance of man vector contact more for Jhumias
- ✔ Presence of Forest and Forest fringe vectors like *An baimaii, An minimus, An maculatus* - *LLIN not sufficient*
- ✔ Pre-bedtime, outdoor and early biting- *LLIN not sufficient*
- ✔ Highly potent and highly anthropophilic vectors
- ✔ No indoor resting found- IRS *not sufficient*
- ✔ Forest related activities- *LLIN & IRS not sufficient*
- ✔ Hard-to-reach – *IRS difficult, LLIN distribution too*

Need for additional tailor made interventions targeted for Jhumias and MultiPronged approach to research

Additonal Tailormade Interventions *Targeting Jhumias*

Second ary vectors

Forest Vectors

Forest Fringe vectors

High & prolonged rainfall season

Hard-to-Reach

Outdoor biting difficult to contain by only LLIN & IRS

High anthropophilicity

High Forest Cover

Pre bed time biting- difficult to contain by only LLIN

Persistent Hot Foci

Forest stay & associated activities

Problem of Health system surveillance and delivery

Exophillic – difficult to contain by only IRS

Near to Mynamar border

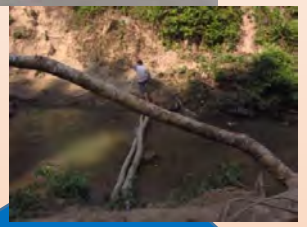
Carriers of resistant Pf strain ?

Poor KAP related to Malaria

Circulation of drug resistant strains

High hidden asymptom atic load

Low SES



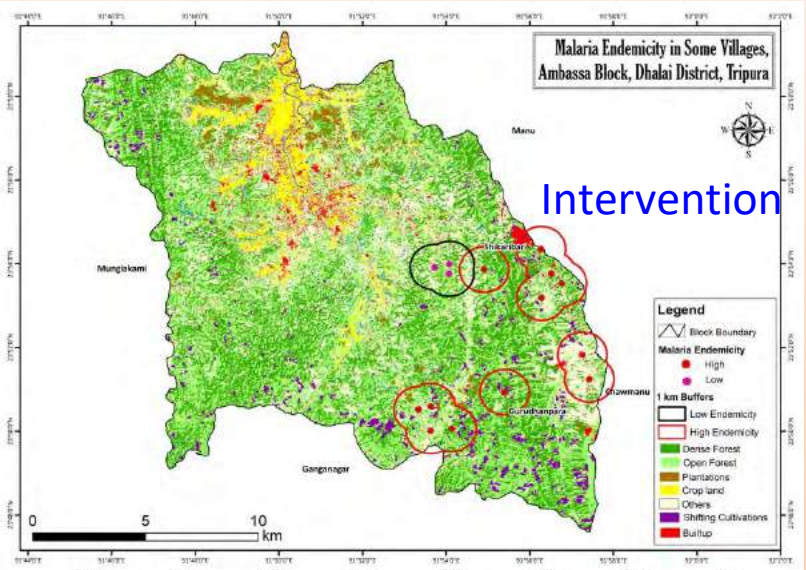
Additional Tailor-made Targeted Intervention Package designed to address the gaps:

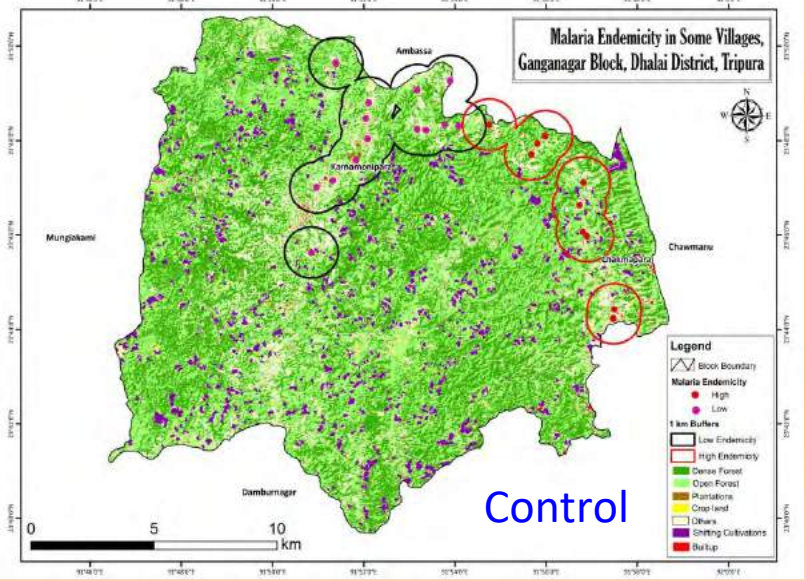
- ✔ Village volunteers trained & recruited
- ✔ Repellent cream distribution, IEC and monitoring
- ✔ IEC & constant monitoring for better coverage , compliance and usage of LLIN
- ✔ App assisted real time surveillance
- ✔ High coverage mass survey among Jhumias
- ✔ Modified IEC for better surveillance and control through village based volunteers

Study Area

Dist: Dhalai

PHCs: Ambassa, Ganganagar





Study design

Intervention given in 2 Subcentres and 2 kept as control in both very high and moderately high endemic areas

Very High Endemic area

- Intervention
- Gurudhan SC :7 villages.
- Pop ~2000, Mostly Tripuri Tribe
- 2016-17 API ~ 138

- Control
- Maldapara SC : 8 villages
- Pop ~2300. , Mostly Tripuri Tribe
- 2016-17 API ~ 113

Moderately High Endemic area

- Intervention
- Shikaribari SC : 11 villages
- Pop ~ 2000 Mostly Reang Tribe
- 2016-17 API ~ 45

- Control
- Karnamani SC : 17 villages
- Pop ~ 2700 Mostly Reang Tribe
- 2016-17 API ~ 55

- Total population ~9000, 40 villages

Study Analysis

Intervention package started 2019 June onwards

Assessment of intervention impact by comparing intervention and control areas for the following:

- Vector control methods status by physical verification

- Malaria symptomatic case and SPR status using Rolling correlation with window size 12, to understand the dynamic nature correlation trends between the two time-series.

- Moving Weighted Average Difference: T defined as sum([SPRcontrol]i - [SPRintervention]i)/sum([SPRcontrol]i) estimated for moving frames consisting of 12 (summation over 12 points, i = 1 to 12) consecutive data points similar to that of rolling correlation coefficients.

Repellent distribution and IEC among Jhumias




Repellent found in use in Jhum huts



mosquito repellent cream use verification and IEC


Physical verification and feedback surveys were regularly taken

Based in feedback, formulation of DEPA changed to get rid of burning sensation.

Modified time of mass screening to catch the permanent and other Jhumias

To cover the Jhumia people innovative screening schedule and places were used like late afternoon near the shops in the roads where the Jhumias returning are found, house to house evening and night surveys before market days and very early morning survey near car loading market days areas on

Malaria Mass Surveillance Work during Covid -19 time






- ✔ Enormous burden of RDT negative asymptomatic and submicroscopic malaria with *P. vivax* and mixed infections during the mass surveillance in dry winter month, with the evidence of malaria transmission found in a reportedly *P. falciparum* dominating region. (Pathogens. '21)

- ✔ In the 2020 survey, despite the COVID 19 situation and high rate of refusal among people to give blood, specially when without symptoms, mass survey coverage in June-July has been achieved upto 90% in some areas, while others had less than 60% coverage
- ✔ Mass survey coverage, though was quite high in the 1st year till the mid 2020 (even during the 1st lockdown period, a high coverage could be achieved in many of the villages as village volunteers and ASHAs continued the survey), it constantly deteriorated afterwards, specially after the Covid 19 situation.

- ✔ Main culprits: the reluctance of people to give the blood, Covid 19 where people were sceptical about getting tested if they do not have a fever and multiple surveys being conducted in a year (4 times). People were reluctant, especially when they did not have symptoms and could not understand the importance of getting tested in the absence of any sickness.

Old Torn LLIN use in absence of new LLIN, reported and new LLIN given, May,'19

Market net use found instead of LLIN, reported, June,'19

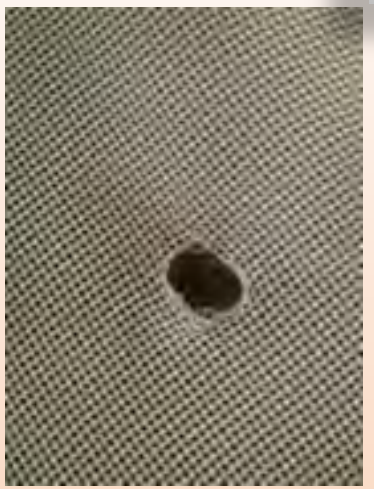
No bednet USE while sleeping, July, '19

LLIN USE while sleeping, July, '19

Successful IEC to convince people to change old LLIN to new LLIN, June , July, '19

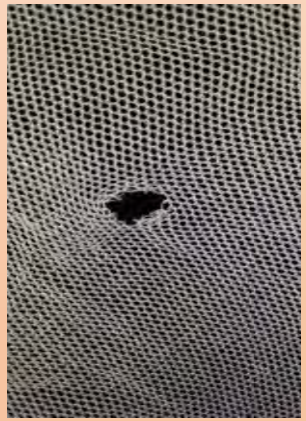
Various Types of Holes found in In LLIN in 7-8 months of usage

Burn hole



Bamboo cut hole

Rodent cut hole



Holes due to paddy sieving

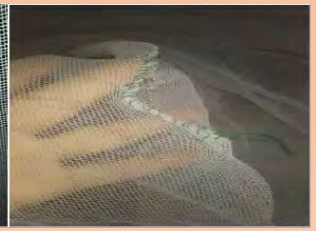
Hole Size	0.5cm to 2 cm	2cm to 10cm	10cm to 25cm	>25cm	Total LLINs
Village					
Dhansing	31	18	5	0	189
Bidhyapara	14	12	13	0	133
Sambhunath	4	2	1	0	25
Sudhiram	0	7	0	0	25

Continuous physical verification of LLIN : Coverage Compliance, Condition monitoring and IECs

Inspection of unused LLINs

New LLIN Distributions in remote Villages where shortage is reported

Before Stitching Teaching Stitching After Stitching



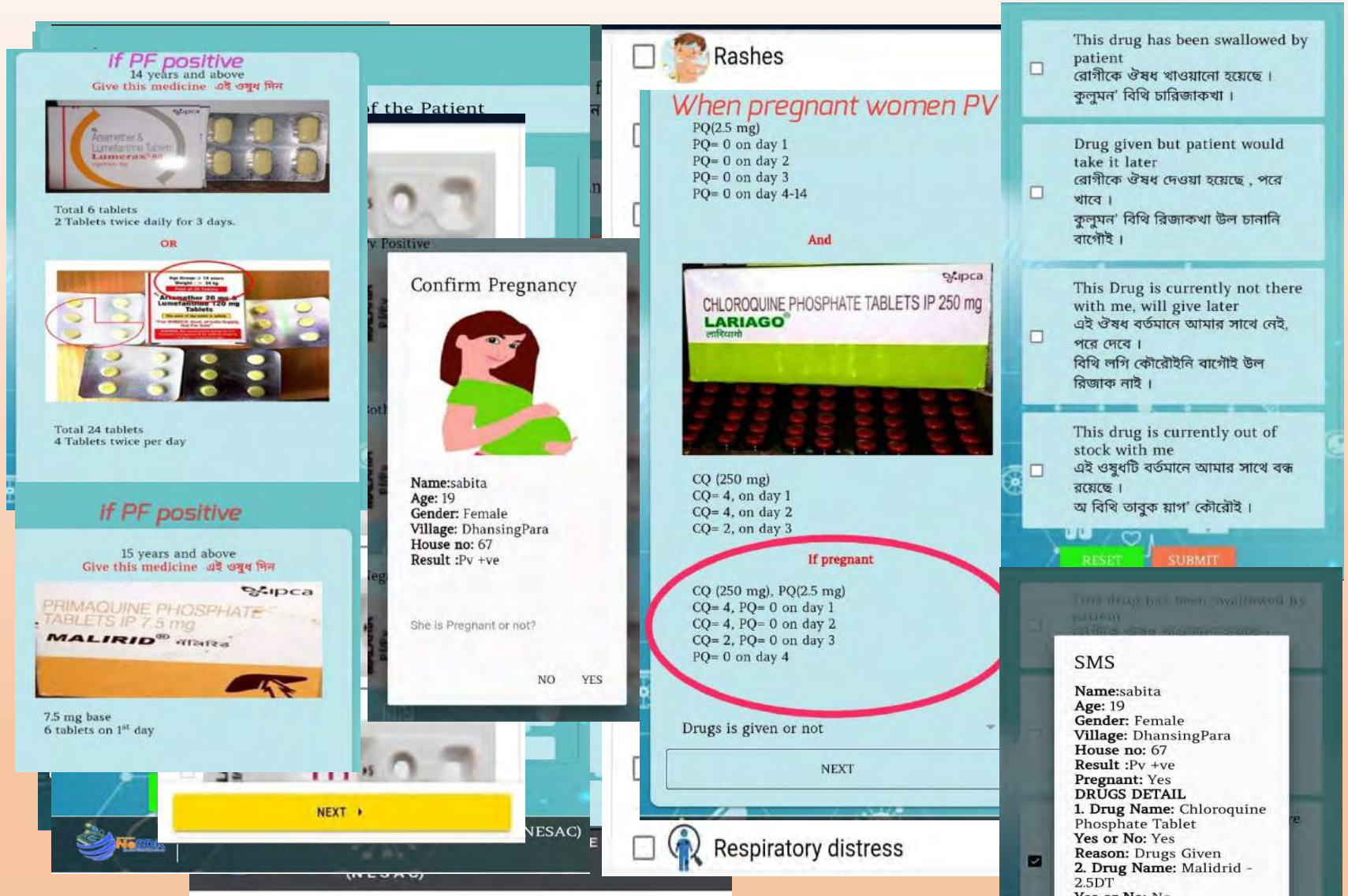
Wrong stitching Corrected stitching

High LLIN coverage and compliance were found, however, several issues regarding the quality and condition found, which is leading to non-usability of the LLINs

A pictorial multilingual app augmenting the real-time surveillance for fever and specifically Malaria named '***FeverTracker for Health Workers***

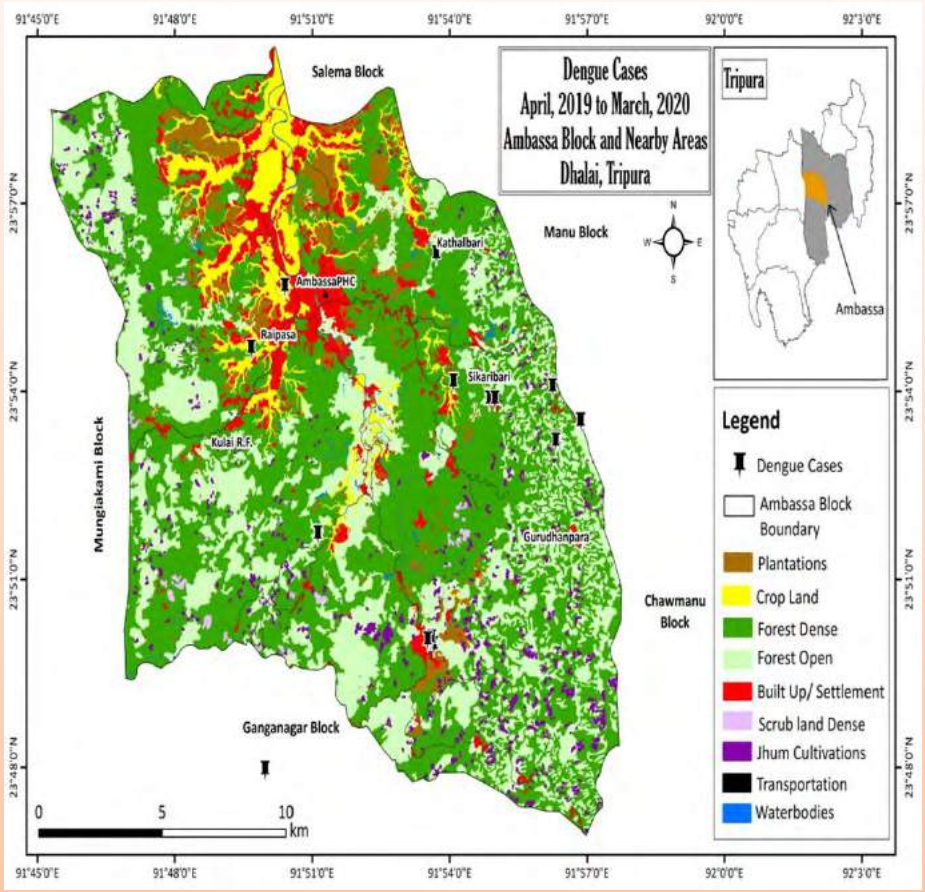
Accurate User-friendly Surveillance at all possible levels, Accurate Drug Dose Guidance & Monitoring

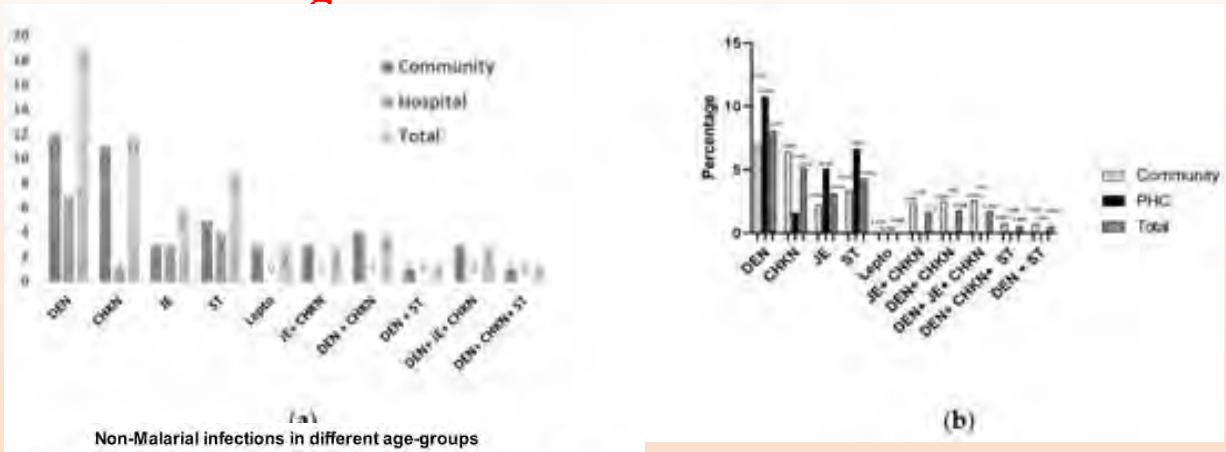
- ✔ All possible surveillances are covered including BSF, CRPF, Private tests
- ✔ Village database of villagers name , HH name , age created , any such database can be integrated -
- ✔ Minimum typing, encourages users to use, Accuracy of information, Same spelling to spot doubling- can be used for any disease surveillance and program
- ✔ All possible symptoms shown as pictorially choices in local language
- ✔ Dengue, Chikungunya, JE,Scrub Flu, Covid19 symptoms also to help assess situation in remote malaria endemic areas - can help in Integrated fever surveillance

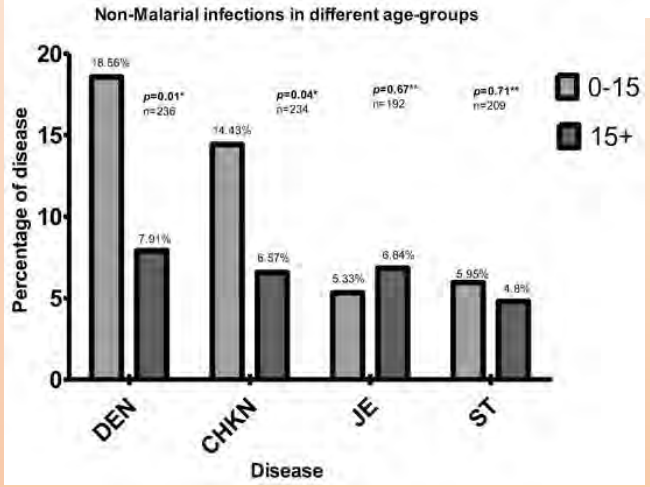


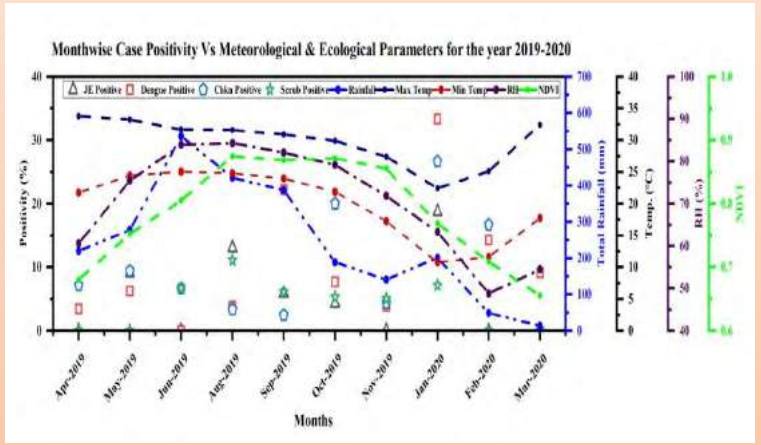
Bhowmick IP et al , JMIR Formative Research, 2021
Copyright obtained , Full Patent Filed
ICMR Intramural Innovation Award, 2023

Presence of other vector borne diseases in high malaria endemic forested areas





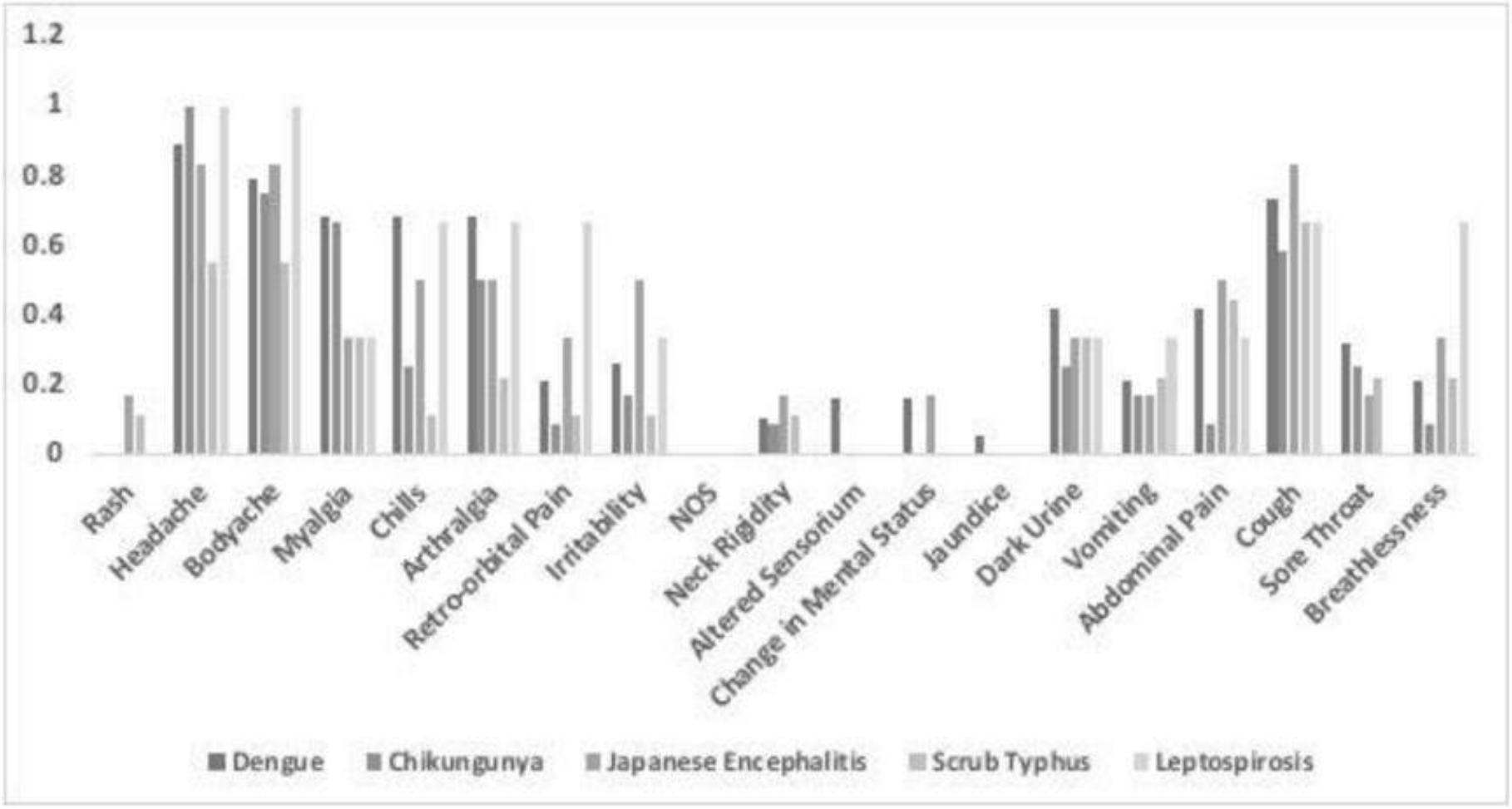




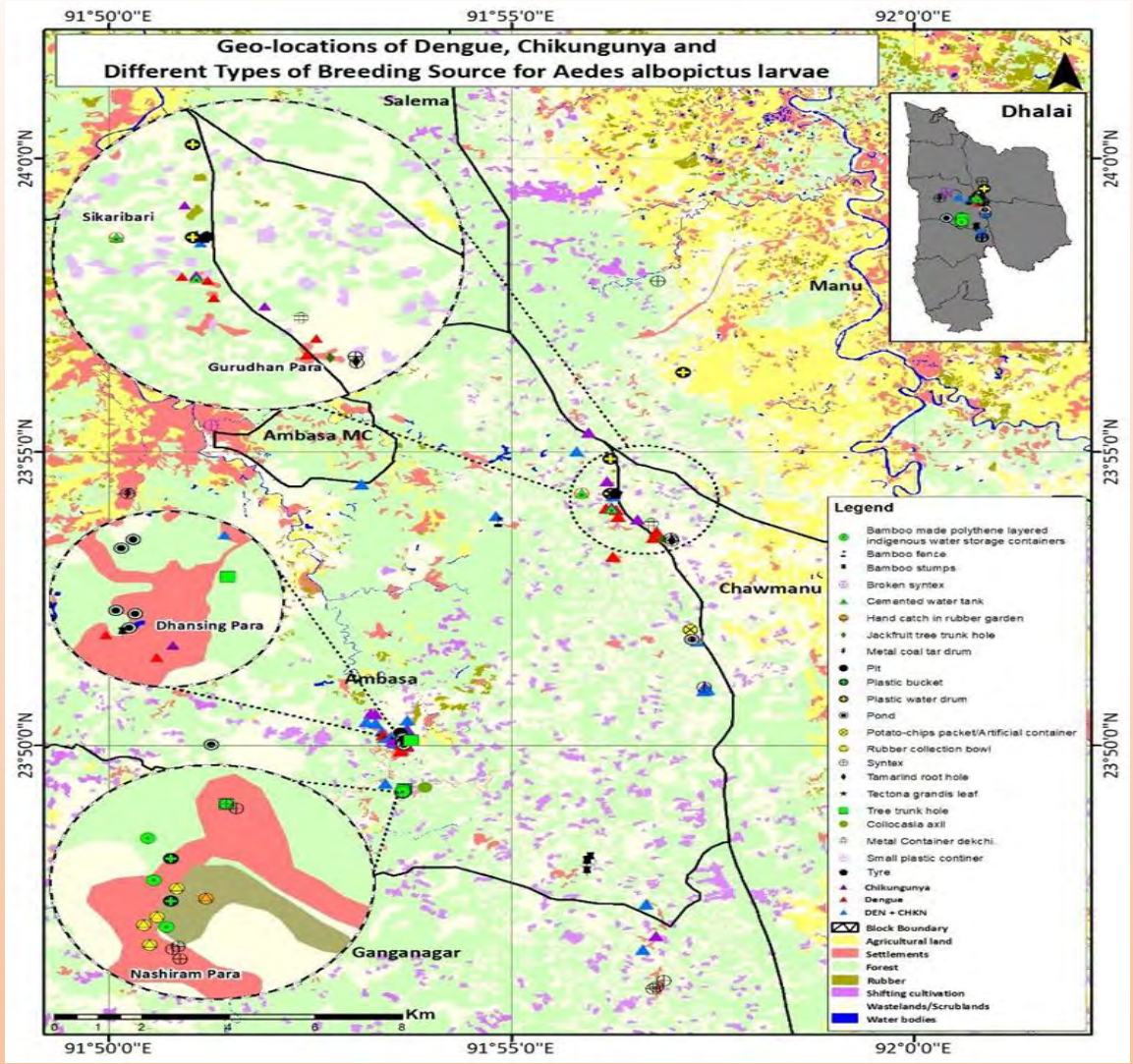
For the first time, diagnosis of indigenous non-malarial vector-borne infections like Dengue, JE, Chikungunya, Scrub Typhus and Leptospirosis found from community and rural hospital surveillance in a high malaria-endemic forested Tribal villages of Tripura- Year long transmission, age as risk factor and vector found. **Bhowmick IP et al, Diagnostics , 2022**

For the first time several co-infections of vector borne diseases with Malaria found in Tripura

Overlap of symptoms in different vector borne diseases



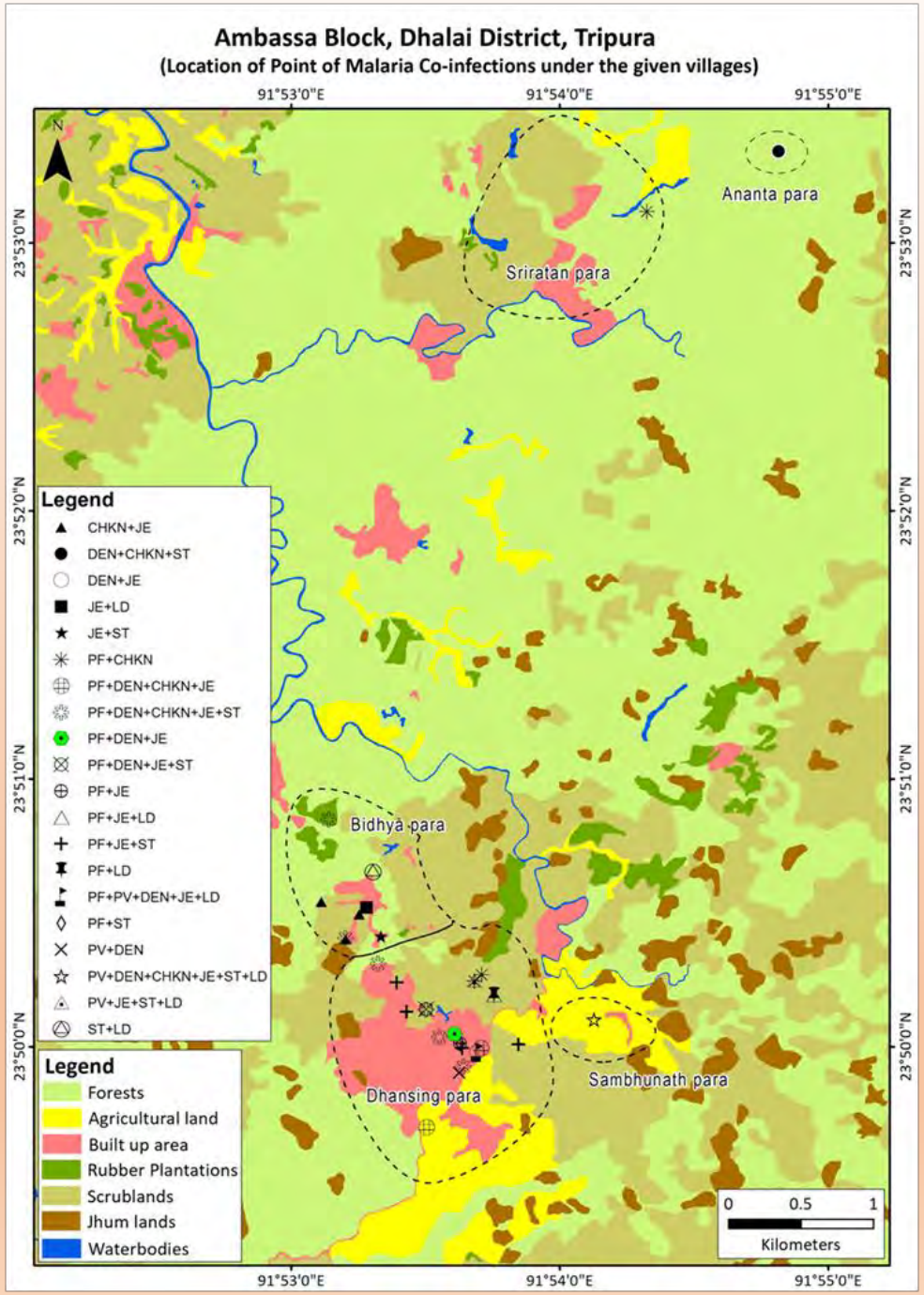
Bhowmick IP et al, Diagnostics 2022



Dengue cases and vectors
found near to rubber garden areas

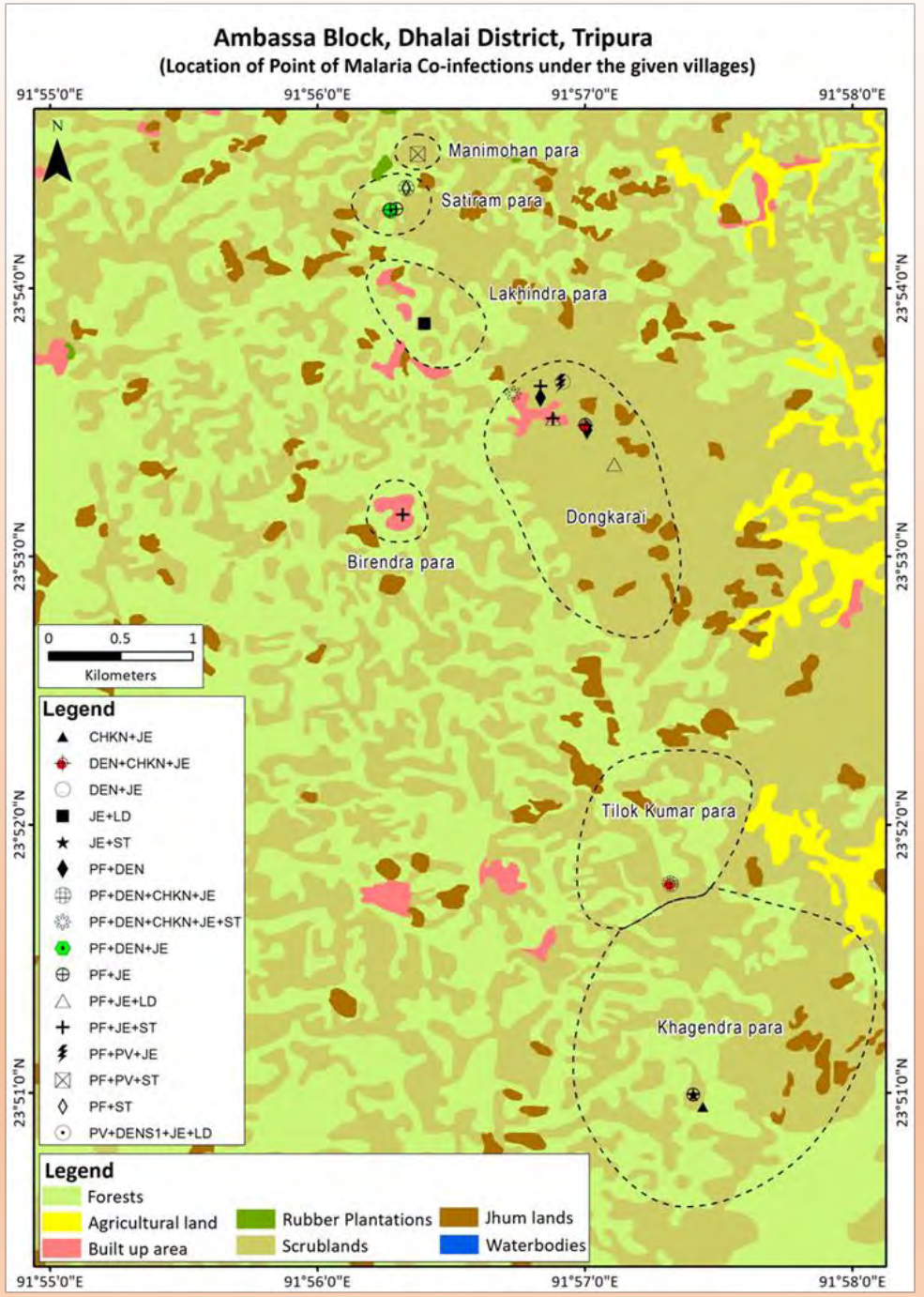
LULC map showing the geolocation of the Chikungunya and Dengue cases along with the positive container locations. Some areas are zoomed in to show the rubber garden and forested area nearby.

Ref: Biomedicines doi: 10.3390/biomedicines11082186.



Malaria co-infections

MS Submitted



2014 Post-outbreak winter mass survey in Dhalai- revealed huge asymptomatic and submicroscopic burden

MESA ICEMR Study

- A total of 583 individuals were enrolled in the study.
- 416 samples were collected for malaria diagnosis irrespective of their symptoms , tested by RDT, and/or microscopy/PCR.
- 252 samples were tested for malaria by all RDT, microscopy and PCR.

✔ Detected malaria burden was 52.7% of which 34.1% was asymptomatic.

✔ In both the cases *P. falciparum* were dominant with 34.1% and 32.5% respectively over *P. vivax* of 14.7% and 11.1%.

✔ A high burden of submicroscopic and low density malaria was also

✔ detected, 38.9% and 40.5% respectively, *P. falciparum* dominant with 24.2% and 26.2%.

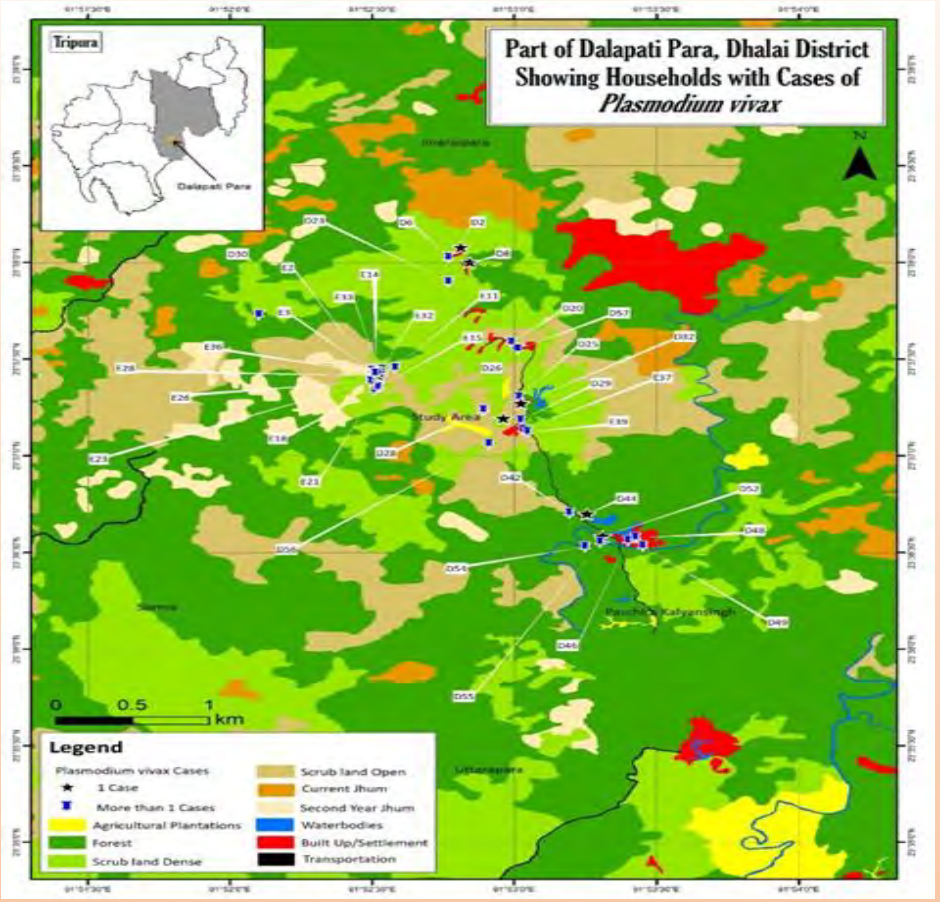


Fig: Map of study area

✔ The proportion of *P. vivax* infections was 6.25%, 8.5%, 7.3%, and **27.6 %** of total cases detected by RDT, microscopy, nested PCR, and nested qPCR respectively making the overall *P. vivax* to be 18.3% of all cases found by any kind of diagnostic methods.

More sensitive methods are able to detect large % of Pv asymptomatic, submicroscopic infections

Sensitivity of the methods used in the present study

Microscopy revealed 72.2% (n=96) total malaria infection in the study and RDT detected 36.0% (n=48). RDT was tested for symptomatic individuals only (n=88). *Plasmodium* genus specific qPCR detected 96.2% (n=128) and species specific nqPCR also detected 96.2% (n=128) malaria. Conventional nPCR could detect only 3% (n=4) of total malaria.

Table: Comparison of Sensitivity by RDT, microscopy, nPCR and qPCR used in the present study

Diagnostic method	**Total malaria %**	**Symptomatic %**	**Asymptomatic %**
RDT*	36.0 (n=48)	36.0 (n=48)	N/A
Microscopy	72.2 (n=96)	43.6 (n=58)	28.6 (n=38)
nPCR	3 (n=4)	1.5 (n=2)	1.5 (n=2)
genus qPCR**	96.2 (n=128)	10.5 (n=14)	84.2 (n=112)
species nqPCR**	96.2 (n=128)	12.0 (n=16)	82.7 (n=110)

Very High Prevalence of *P. vivax* submicroscopic and asymptomatic cases : Feb-March mass survey '21

Enormous burden of RDT negative asymptomatic & submicroscopic malaria with *P. vivax* & mixed infections during the mass surveillance in dry winter month, in a reportedly *P. falciparum* dominating region.

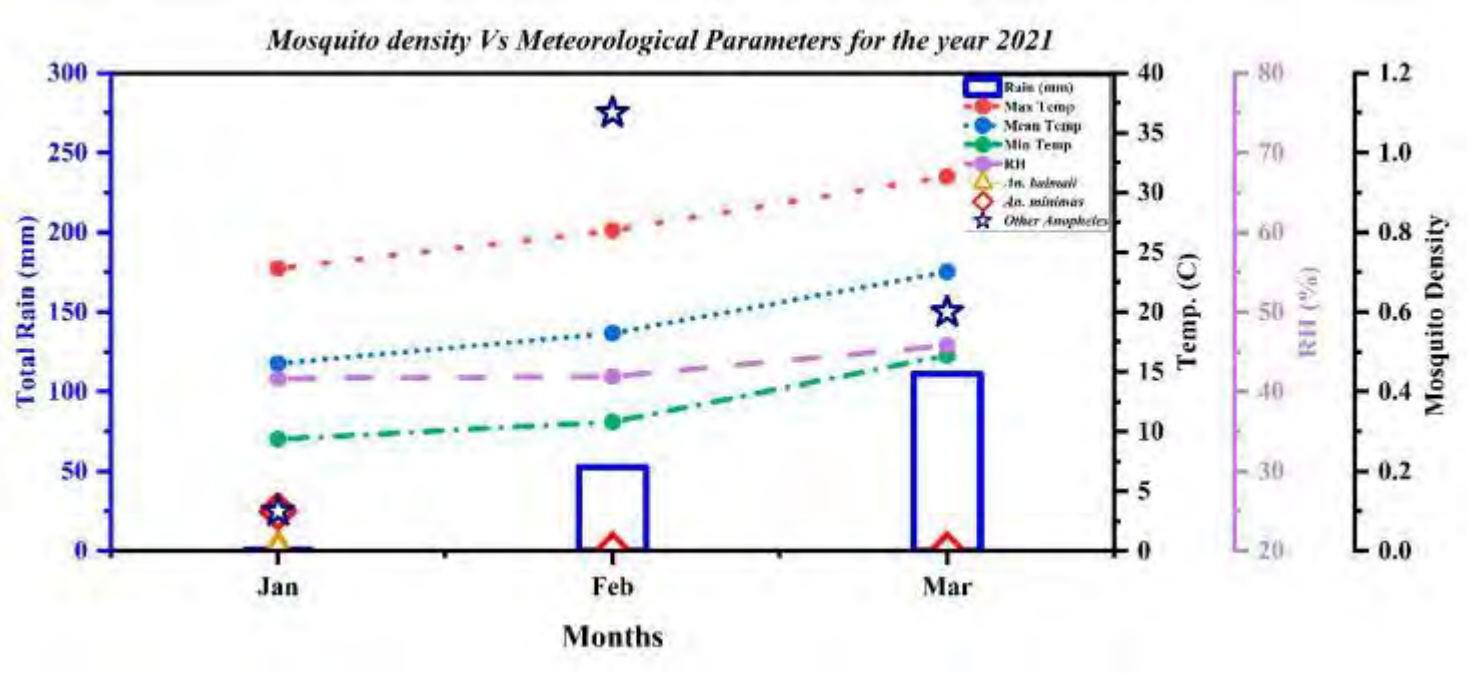
(Bhowmick IP et al, Pathogens. '21)

	Dhansinghpara (N=90) (% positive)	Bidyapara (N=60)(% positive)	Total Positivity (N=150) (% positive)
Pf	5 (5.5)	1 (1.6)	6(4)
Pv	**29 (32.2)**	**11 (18.3)**	**40(26.7)**
Pf + Pv	13 (14.4)	13 (21.7)	26(17.3)
Total	47 (52.2)	25 (41.7)	72(48.0)

RDT missed several Pv cases in the range of 80-400 parasites/µL

Age groups of tested people in two villages	Symptomatic Malaria Cases				Asymptomatic Malaria cases				Total Tested	Positives (%)	Negatives (%)
	Pf (%)	Pv (%)	Pv+Pf (%)	Total Cases (%)	Pf (%)	Pv (%)	Pv+Pf (%)	Total Cases (%)			
0-<5y	0 (0)	1 (9.1)	0 (0)	1 (9.1)	0 (0)	4 (36.4)	1 (9.1)	**5 (45.5)**	11	6(54.5)	5 (45.5)
5 to 15y	0 (0)	1 (1.6)	3 (4.7)	4 (6.3)	3 (4.68)	14 (21.9)	9 (14)	**26(40.6)**	64	30 (46.8)	34 (53.1)
>15y	0 (0)	2 (2.7)	0 (0)	2 (2.7)	3 (4)	18 (24)	13 (17.3)	**34 (45.3)**	75	36(48)	39 (52)
Total	0 (0)	4 (2.7)	3 (4.7)	7 (4.7)	6 (4)	36 (24)	23 (15.3)	**65 (43.3)**	150	72(48)	78 (52)

Age Groups of Tested People in Two Villages	Sub-Microscopic Cases				
	Pf (%)	*Pv* (%)	*Pf* + *Pv* (%)	Total Cases (%)	Total Tested
0 to <5 years	0 (0)	3 (30)	1 (10.0)	4 (40)	10
5 to 15 years	1 (1.8)	14 (25.5)	4 (7.3)	19 (34.5)	55
>15 years	3 (6.1)	7 (14.3)	9 (18.4)	19 (38.8)	49
Total	4 (3.5)	23 (20.2)	15 (13.2)	42 (36.9)	114



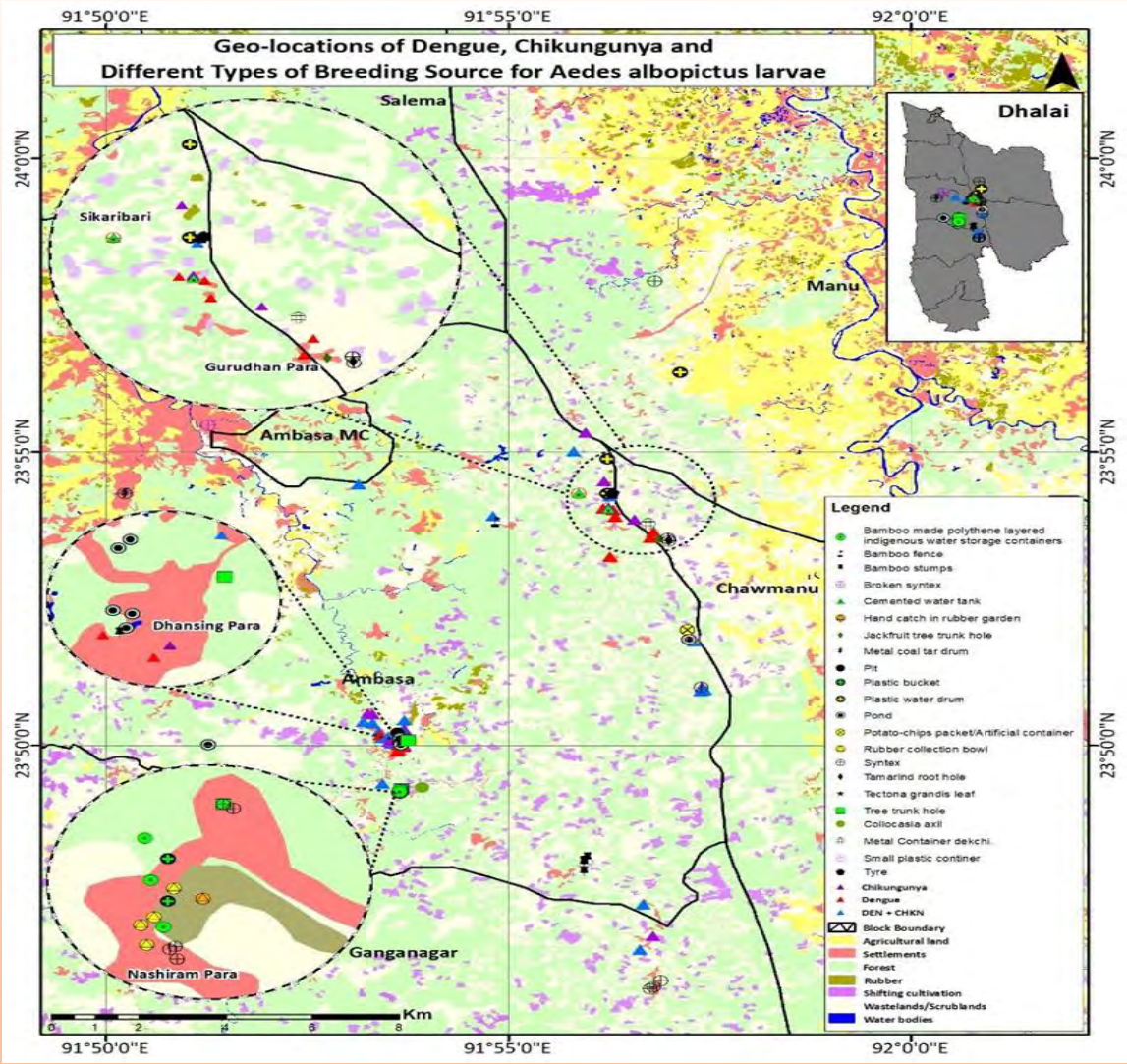
Evidence of malaria transmission found in dry winter months with parasite positivity in Anophele

Gametocytes found in several asymptomatic cases of both Pf, Pv and mixed cases in the range of 53-986 parasites/ul showing the transmission capability

Major Recent Findings

- ✓ In 2022, a huge increase in *P. vivax* symptomatic cases found in the traditional P. falciparum dominated area. Several repeat cases found even after drug compliance and drug resistance to *P. vivax* is found. No drug resistance to the drugs in use now and earlier for of *P. falciparum* found so far.
- ✓ Very high burden of both symptomatic and asymptomatic *P. malariae* reported from the area for the first time, confirmed by microscopy and PCR
- ✓ Jhum cultivation has been found as the major risk factor for malaria in Dhalai, Tripura
- ✓ Repellent creams as intervention has been found to be protecting Jhum cultivators against malaria
- ✓ Additional intervention package targeted for Jhum cultivators has been able to have impact on malaria control
- ✓ Dengue, Chilkungunya, JE, Scrub typhus, Lepotospirosis and Lyme disease along with co-infections with *P. falciuparum* and *P. vivax* reported for the first time, among the Tribals in the forested regions.

Entomological Studies in Tripura and different parts of NE India: Vectors other than known An baimaii, An minimus found



Dengue cases and vectors
found near to rubber garden areas

LULC map showing the geolocation of the Chikungunya and Dengue cases along with the positive container locations. Some areas are zoomed in to show the rubber garden and forested area nearby.

Ref: Biomedicines doi: 10.3390/biomedicines11082186.

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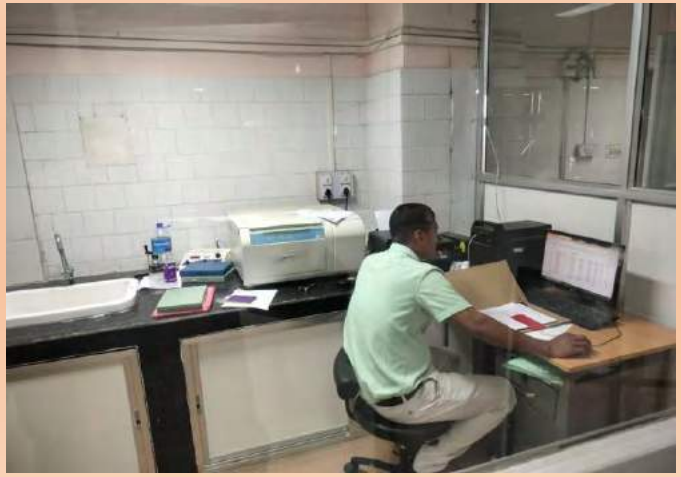

Tripura

South Tripura District- Baishnabpur PHC Area and North Tripura District- Anandabazar CHC Area
















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Village volunteers at Tripura

Nansha Tripura
Khullojoy Tripura
Mathunjoy Tripura
Chiranjoy Tripura
Gaganjoy Tripura
Babulsha Tripura
Khalendra Reang
Gapendra Reang
Tangkarai Reang
Kinadhan Tripura
Labadha Tripura
Santu Joy Tripura
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