9. SUMMARY
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9.1 Introduction

North-Eastern region of India provides congenial climate for mosquito proliferation and malaria transmission which is uninterruptedly supported by many efficient Anopheles vectors annually. In this region three species of human malaria parasite, namely Plasmodium falciparum, P. vivax and P. malariae however P. falciparum is more common and accounts for > 60% of the total malaria infections. Various components such as parasite load in the population, prevalence of potential vector and adequate host vector interaction are main contributing factors for high malaria incidences.

Immunochromatographic rapid diagnostic test kits are very useful for on the spot detection of malaria parasite. However polymerase chain reaction assays have been proved more sensitive to detect malaria parasite at species level. Xenomonitoring of malaria in vector mosquitoes and host preference are important epidemiological aspects in malaria transmission. The data on host preference and incrimination of certain malaria vectors which appear in large density is still scanty in the studied region. Present research was undertaken in some malaria endemic areas to identify the prevailing malaria parasite to assess the utility of different diagnostic methods in malaria detection, to identify the potential malaria vectors and to collect information on insecticide susceptibility of malaria vectors.

9.2 Methodology

Adult Anopheles mosquitoes were collected from dusk to dawn using CDC traps and identified morpho-taxonomically. Malaria investigation was carried out using the active malaria survey by collection of thick and thin blood smears on glass slides using
finger prick method and examining them under microscope. A cohort of malaria samples collected from an endemic area was used to compare the efficacy of rapid diagnostic kit and PCR assay to microscopy. The insecticide bioassay for DDT and deltamethrin was performed following the WHO standard testing that involved tarsal contact exposure to insecticide impregnated papers.

9.3 Malaria epidemiology

Along Indo-Bangladesh border in South Tripura 1043 blood smears were examined of which 269 were found malaria positive (Slide Positivity Rate = 25.8; SFR = 22.2; Slide Vivax Rate = 3.1). The children of 2-4 years age group were worst affected (Slide Falciparum Rate = 58.3; SVR = 6.0). SFR between the different age groups was statistically different ($\chi^2 = 56.9; p < 0.001$) while SVR was similar among the different age groups ($\chi^2 = 5.9; p = 0.4$). The difference between $P. falciparum$ and $P. vivax$ percent parasitemia during different seasons was found to be statistically significant ($t = 3.7; p = 0.0003$ for $P. falciparum$; $t = 2.2; p = 0.03$ for $P. vivax$). In Dhalai, 346 samples were collected from the three TSR units located at Kakchangamin, Lalcherra and Kacharichera (SPR = 27.7; SFR = 23.4). In the villages surrounding TSR units 210 blood samples were collected (SPR = 51.4%; SFR = 46.7%). There was no significant difference in the malaria incidence among three TSR units ($\chi^2 = 4.0; p = 0.1$). Whereas the malaria incidence was significantly higher in Kacharichera in comparison to the other two villages ($\chi^2 = 23.6; p < 0.0001$). The parasitemia among TSR personnel was higher than the villagers ($t = 4.7; p < 0.0001$). Out of 1182 blood smears examined in seven tea estates of Assam, 506 were found to be positive for malaria infection (SPR = 42.8). Dimakuchi tea estate was found to have highest SPR ($\chi^2 = 14.2; p = 0.03$) than the other tea estates. Highest (154) malaria positive cases were detected in humans
having O(+ve) blood group but did not differ statistically ($p = 0.3$). The children below two years of age were found to be most affected ($p = 0.002$) with high SPR of 60. Haemoglobin content in malaria infected males and females were found to be significantly lower compared to their normal counterparts ($p < 0.0001$).

### 9.4 Anopheline vectors

A total of 6771 adult mosquitoes of known anopheline vector species were collected from 26 study sites of 7 different areas in 205 trap nights (per night trap density = 33.0) during 2009-12 belonging to 16 species of *Anopheles* namely *An. dirus*, *An. fluviatilis*, *An. annularis*, *An. culicifacies*, *An. minimus*, *An. vagus*, *An. varuna*, *An. philippinensis*, *An. nivipes*, *An. subpictus*, *An. jami*, *An. splendidus*, *An. maculatus*, *An. kochi*, *An. aconitus* and *An. karwari* were recorded in the current study.

In South Tripura and Dhalai along the Indo-Bangladesh border *An. vagus*, *An. philippinensis*, *An. nivipes* and *An. minimus* were the predominant species and constituted 36.6, 18.8, 25.2 and 12.6% respectively ($\chi^2 = 5508.5; p < 0.0001$). While in Assam-Meghalaya border in Khasi hills *An. philippinensis/nivipes*, *An. annularis* and *An. vagus* were prevalent in large number with a percentage of 17.5, 17.1 and 15.6 respectively whereas *An. jeyporiensis* (10.6%), *An. karwari* (10.8%) and *An. jami* (7.5%) were found in comparatively lesser number.

Along the Indo-Bhutan border in Tamulpur *An. philippinensis/nivipes* (66.9%) were recorded in highest number. *An. minimus* was sparsely distributed in the area and contributed 6.6% of total collection. The per trap night density was highest in *An. philippinensis/nivipes* (66.1) and lowest in *An. annularis* (2.3).

In Tezpur military station of Assam, *An. vagus* was predominant species constituting 64.1% of the total collection ($\chi^2 = 740.1; p < 0.0001$). *An. minimus* and *An.
*culicifacies* constituted 6.0 and 15.5% respectively with a per trap night density of 0.4 and 1.1 respectively. *An. philippinensis* and *An. annularis* were the other vector species sharing 5.6 and 7.2% respectively. In Missamari four major malaria vectors, namely *An. minimus, An. dirus, An. culicifacies* and *An. fluviatilis* were recorded of which *An. minimus* and *An. fluviatilis* were predominantly distributed ($\chi^2 = 86.1; p < 0.0001$). Among the five secondary malaria vectors *An. philippinensis* and *An. varuna* were predominant and recorded 33.1 and 26.0% respectively ($\chi^2 = 113.1; p < 0.0001$). In Sibsagar (upper Assam) *An. philippinensis* was major species (54.2%) ($\chi^2 = 381.5; p < 0.0001$) whereas *An. annularis* and *An. culicifacies* were recorded in low number during the study period. In Dinjan (upper Assam), *An. annularis* was the main species contributing 64.3% ($\chi^2 = 498.3; p < 0.0001$) while *An. maculatus* was contributing only 3.1% of the total collection.

Study on ITS-2 sequences of *An. vagus, An. varuna* and *An. jeyporiensis* displayed maximum intraspecific similarity. Genetic distance range in *An. vagus, An. varuna* and *An. jeyporiensis* was 0.0180-0.0020, 0.0151-0.0528 and 0.2520-0.3314 respectively. The overall analysis revealed that intraspecific genetic distances did not vary considerably and show the maximum similarity.

### 9.5 Xenomonitoring and human host preference

In Tezpur (Assam), the anthropophilic index (AI) of *An. minimus, An. culicifacies, An. annularis* and *An. philippinensis/nivipes* was found to be 90, 37.3, 26.9 and 45.9 respectively ($\chi^2 = 15.3; p = 0.002$) while in Chandubi (Assam) AI was 20.8. *An. minimus* was recorded in lesser number but its AI was higher compared to *An. annularis* and *An. vagus*. 

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Only 7 specimens, two each of *An. culicifacies* and *An. annularis* and three of *An. minimus* were detected positive for the presence of human malaria parasite in Tezpur. The nested PCR assay revealed that all three (100%) *Plasmodium* positive *An. minimus* mosquitoes were harbouring only *P. falciparum* whereas one each specimen of *An. annularis* and *An. culicifacies* (50%) was found to be positive for *P. falciparum*. In Chandubi only one *An. annularis* specimen was found to be positive for *P. falciparum*.

### 9.6 *Plasmodium* species diagnosis

Sensitivity and specificity of OptiMal-IT® rapid malaria detection kit as compared to microscopy was found to be 93.1% and 83.3% respectively whereas its overall performance for malaria diagnosis was 76.4%. The sensitivity and specificity of PCR assay in detecting malaria parasite was found to be 96.6% and 100% respectively whereas the overall performance was 96.6%. The OptiMal-IT® rapid malaria detection kit detected 4 false negative and 3 false positive therefore its performance dropped down significantly. Nested PCR assay correctly identified the *P. malariae* which were misidentified as *P. falciparum* by microscopy. PCR also identified three *P. falciparum* and *P. malariae* mixed infection and one *P. malariae* mono-infection.

### 9.7 Insecticides susceptibility of malaria vectors

In South Tripura mortality in *An. minimus, An. varuna* and *An. philippinensis/nivipes* ranged between 80-98% for DDT indicating suspected resistance whereas *An. maculatus, An. vagus, An. kochi, An. aconitus* and *An. annularis* were found to have high level of DDT resistance. Deltamethrin was 100% effective against *An. minimus, An. philippinensis/nivipes, An. kochi* and *An. varuna* whereas resistance was suspected among *An. maculatus, An. vagus, An. aconitus* and *An. annularis* as the corrected mortality was found to be < 98%.
In Assam-Meghalaya border area in Khasi hills An. minimus and An. philippinensis/nivipes were suspected to have developed resistance against DDT (corrected mortality % - 92.5 and 86.0 respectively). An. vagus, An. subpictus, An. annularis, An. jamesi, An. jeyporiensis, An. kochi and An. karwari were found resistant to the DDT. An. minimus, An. kochi, An. karwari and An. philippinensis/nivipes were fully susceptible while An. vagus, An. subpictus, An. annularis, An. jamesi were found to have low level of resistance against deltamethrin.

In Indo-Bhutan border at Tamulpur area An. vagus and An. annularis were resistant while An. minimus, An. philippinensis/nivipes and An. varuna were suspected to be DDT resistant. An. vagus was found to have a low level of resistance to deltamethrin.

In Tezpur (Assam) An. vagus, An. annularis and An. culicifacies were resistant to DDT whereas An. annularis and An. culicifacies were found to develop low level of deltamethrin resistance. It was found that at Dinjan all the tested anopheline species have developed resistance to DDT but completely susceptible to deltamethrin. In Sibsagar (upper Assam) also anopheline species namely An. philippinensis/nivipes, An. annularis, An. culicifacies and An. vagus were found to be resistant to DDT. Knock-down percentage in An. annularis post 10 minutes exposure to DDT and deltamethrin in both Chandubi and Rani areas was similar (\( t = 0.4; p = 0.7 \) for DDT and \( t = 0.7; p = 0.5 \) for deltamethrin). The KDT\(_{50}\) values for both DDT and deltamethrin did not differ among both the study sites (\( t = 0.8; p = 0.4 \) for DDT; \( t = 0.6; p = 0.6 \) for deltamethrin). Probit model used to estimate KDT\(_{50}\) and KDT\(_{95}\) values displayed the normal distribution of percentage knock-down (\( p \geq 0.4 \)). An. annularis preferred resting on non-insecticide sprayed areas as compared to the sprayed areas (\( \chi^2 = 57.8; p < 0.0001 \)).

The present thesis contains 346 references, 40 figures and 29 tables.