MALARIA EPIDEMIOLOGY ALONG INDO-BANGLADESH BORDER IN TRIPURA STATE, INDIA

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Abstract. Malaria epidemiological surveys were conducted in 16 villages along the Indo-Bangladesh border in Tripura, northeastern India. Insecticide resistance among malaria vectors and chloroquine resistance in the parasite were also studied along with monitoring of vector density using light traps. The epidemiological data indicated that malaria incidence was highest during June-July and lowest during November. Examination of blood smears collected through door to door surveys indicated slide positivity rate (SPR) of 25.2% and that Plasmodium falciparum was the predominant parasite (slide falciparum rate of 22.3%). The incidence rates of falciparum malaria varied significantly among the age groups (p<0.001) and 2-4 year olds were the most affected. Major malaria vectors recorded in light trap collections were An. dirus, An. minimus and An. philippinensis/nivipes. Chloroquine resistance studies indicated that treatment failure occurred in 35% of the cases and hence the use of artesunate combination therapy (ACT) was recommended for treatment of malaria in the area.

Key words: malaria, epidemiology, vector, resistance, Tripura, India

INTRODUCTION

Approximately 247 million cases of malaria are reported annually from 109 countries; 3.3 billion people are estimated to be at the risk of contracting the disease (WHO, 2008). In India, malaria is endemic in most parts where insecticide resistance among malaria vectors, drug resistance in malaria parasites and lack of adequate resources pose serious challenges for fighting malaria (Sharma, 2003; Bhattacharya et al, 2006). Nine species of anopheline mosquitoes are responsible for malaria transmission in India of which six are of primary importance (Raghavendra and Subbarao, 2002). Development of chloroquine resistance in Plasmodium falciparum, first reported from the northeastern state of Assam (Sehgal et al, 1973), has emerged as a serious challenge in combating malaria in India. Systematic monitoring of drug resistance is undertaken by the National Vector Borne Disease Control Programme (NVBDCP) and the latest studies indicate that chloroquine resistance in P. falciparum is widespread in the country (Arora et al, 2008). Although resistance to chloroquine has been reported in P. vivax, it is still effectively used for the treatment of vivax malaria, which contributes 60-70% of malaria cases in India (Dua et al, 1996; Shah, 2008). A few cases of resistance to other antimalarial drugs like sulfadoxine have also been reported
from the country (Dua et al, 2003).

The transmission of malaria in northeastern India presents a formidable epidemiological challenge. The distribution of the disease in the region is heterogenous and the dynamics and intensity are governed by numerous biological and other factors (Dutta et al, 2004). Focal outbreaks of malaria are common in this region, which accounts for 8-12% of all the reported malaria cases in India. *Plasmodium falciparum* is a major malaria parasite in this region, causing 60-80% of malaria infections. The transmission of the disease is facilitated by the vectors *Anopheles minimus*, *An. fluviatilis* and *An. dirus*. The hot and humid climate prevailing in the region is ideal for the survival and multiplication of malaria vectors (Dev et al, 2003; Mohapatra et al, 2008). There is a possibility for multi-drug resistant falciparum malaria, prevalent in Myanmar and Thailand, entering India through the northeastern states (Dua et al, 2003), which warrants monitoring of status of drug resistance in this part of the country.

The regions adjacent to the Indo-Bangladesh border are mostly covered with thick forests and have poor communication and health infrastructure. Armed forces and paramilitary personnel serving in the area have low levels of immunity and are highly vulnerable to malaria (Dutta et al, 2004). Tripura is one of the states in the northeastern region of India, which shares a long international border with Bangladesh. The hilly and undulating terrain and the movement of people across the border have led to a persistence of malaria in the villages near the border. However, knowledge on the bionomics of malaria vectors and the dynamics of malaria transmission in this region is largely inadequate (Prakash et al, 1998). The present study was undertaken to understand the incidence and transmission of malaria in the Belonia Subdivision of Tripura State, India with special emphasis on the villages along the Indo-Bangladesh border. The extent of chloroquine resistance in the malaria parasite and insecticide resistance in major malaria vectors were also investigated.

MATERIALS AND METHODS

Study area

South Tripura District (latitude 22°56’ to 24°32’ N, longitude 91°59’ to 92°22’ E) has five administrative units with a total population of 0.79 million (45% ethnic tribes) and 2,625 km² area (Fig 1). The district has a thick forest cover (76% of total area) and shares a 320 km long border with Bangladesh. The climate is hot and humid with mean rainfall 222.6 cm, mean relative humidity 90% and the temperature ranges from 7°C in winter to 38°C in pre-monsoon. The Belonia Subdivision (population = 0.26 million) has about 70 villages, most of which are along the Indo-Bangladesh border.

The villages selected for the study were surrounded by paddy fields, betel cultivation sheds, ponds and there was a river flowing along the international border. The villages were inhabited mainly by local tribes and Bengali community. On an average there were five persons inhabiting each house. Most of the houses were having two to three rooms with mud floor, mud plastered bamboo walls and thatched roofs. The houses had cattle sheds attached to them and were generally located near water bodies.

Malaria control program

The district health authorities undertake malaria control program with the objective of reducing the *P. falciparum* incidence and deaths due to malaria.
Past epidemiology of malaria

The epidemiological data for South Tripura District (2001-2006) were obtained from the Department of Health, Tripura and were analysed (Fig 2). These data were of malaria smears by health workers in the villages (active detection) and from the patients, who visited the health centers (passive detection).

Blood smear survey in the community

Entomological and epidemiological surveys were carried out in pre-monsoon and monsoon seasons during 2007-2008. Active malaria survey was made in sixteen villages near the international border by door to door collection of thick and thin blood smears on glass slides by finger prick method from persons having fever history for the past ten to fourteen days. The blood smears were stained with Giemsa stain and examined under a microscope for malaria parasite. The suspected malaria patients were examined again on the spot by ICT kits (FirstSign™, M/S Unimed Biosystems) for rapid diagnosis of malaria. The patients found positive for malaria parasite were given treatment as per the guidelines of the National Vector Borne Disease Control Programme (NVBDCP, 2007) with the help of a medical practitioner. Smears were confirmed negative for malaria only after screening 100 fields with each field containing 20-50 white blood cells. The slides confirmed negative were mixed with 10% positive slides and were reexamined by a senior.
SPR, Slide positivity rate; SFR, Slide falciparum rate; API, Annual parasitic index; ABER, Annual blood examination rate

Fig 2—Epidemiological situation in South Tripura District, India, 2001-2006 (Source: Chief medical office, South Tripura, India).

Technician. Parasite count per microliter of blood (parasitemia) was calculated as, parasitemia = (No. of parasites / No. of WBC) x 8,000 (Singh et al, 2006) whereas the percent parasitized RBC was determined as (Parasitemia / 4,000,000)* 100 assuming 4,000,000 RBC per microliter of blood and single parasite per RBC.

Drug resistance

The prevalence of chloroquine resistance in Plasmodium falciparum acute uncomplicated malaria was determined in vivo. The standard protocol for assessing the therapeutic efficacy of antimalarial drugs for uncomplicated falciparum malaria (WHO, 1996) was applied. Patients with clinically confirmed P. falciparum infection were enrolled as volunteers for the study after obtaining informed consent. The study was approved by the ethics committee of Defence Research Laboratory, Tezpur, India. The blood slides were stained with Giemsa stain (pH 7.2) and the number of asexual parasites were counted for every 200 white blood cells (WBC). The clinical and parasitological responses were classified as early treatment failure (ETF), late clinical failure (LCF) and adequate clinical and parasitological response (ACPR). The subjects showing treatment failure with chloroquine were subjected to artesunate combination therapy (ACT) as per the existing guidelines, under the supervision of a medical practitioner.

Entomology

CDC light traps were used to monitor the malaria vector population in the villages during 2007-2008. Five villages along the border where the epidemiological surveys were carried out were randomly selected for the monitoring in 2007. The traps were operated during 06:00 PM - 06:00 AM for two consecutive nights in human dwellings in each of the study villages. The study was repeated in another set of five villages in 2008. Per trap night densities of the malaria vectors were determined based on the light trap collections.

Susceptibility tests for DDT and deltamethrin were performed using WHO kits and standard methodology against Anopheles minimus and Anopheles philippinensis/nivipes collected from the houses. The adult mosquitoes were exposed for an hour to paper impregnated with 4% DDT and for 30 minutes to paper impregnated with 0.05% deltamethrin. The mosquitoes exposed to untreated papers served as control. The mortality after 24 hours post-exposure period was recorded and corrected percent mortality was determined using Abbott’s formula.

Data analysis

The annual parasitic index (API) was referred as the total number of malaria positive cases per 1,000 subjects per year.
The annual blood examination rate (ABER) was defined as the blood percent samples examined per year. The slide positivity rate (SPR) was the percent positive cases among the blood samples examined. The proportion of blood smears found positive for *P. falciparum* / *P. vivax* was the slide *P. falciparum* rate (SFR) / the slide *P. vivax* rate (SVR). Chi-square test was used to analyse the difference in malaria incidence among age-groups, sex, seasons and parasite species.

**RESULTS**

**Past epidemiology**

Analysis of epidemiological data for the district during 2001-2006 indicated that the mean (± standard error of mean) ABER was 10.8 ± 0.5 while the mean SPR was 11.8 ± 0.4 (Fig 2). The SFR and API was 9.8 ± 0.6 and 13.6 ± 1.1 whereas the number of deaths per year due to malaria was 5.3 ± 1.1. The month wise SPR in the Belonia, South Tripura (2005-2006) is depicted in Fig 3. The incidence of malaria was found to vary significantly among the months ($\chi^2 = 71.0; \text{df} = 11; p < 0.001$). The highest SPR (13.37%) was recorded during June-July, whereas the lowest SPR (3.55%) in November.

**Epidemiological studies**

A total of 1,117 blood smears were examined from the district of which 282 indicated the presence of the malaria parasite (SPR = 25.2%). The number of *P. falciparum* cases detected was 249 (SFR = 22.3%) while *P. vivax* was detected from 33 samples (SVR = 2.9%). Age wise and season wise data on malaria cases detected in the present study are given in Table 1. Among the 282 malaria cases in the region, 187 were children below 15 years (66.3%). The SFR in the children less than 2 years age was 28.6% while children in the age group 2-4 years were the worst sufferers (SFR = 47.7%). The SFR was 33.2% among 4-10 year old children, while it was 18.9 and 13.7% in 10-15 years and >15 years age groups, respectively. The difference in SFR between the age groups was found to be significant ($\chi^2 = 64.8; \text{df} = 4; p < 0.001$). However, there was no significant difference in the incidence of *P. vivax* malaria between the <10 year
Table 1

<table>
<thead>
<tr>
<th>Age group (year)</th>
<th>Indices</th>
<th>Plasmodium falciparum</th>
<th>Plasmodium vivax</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre-monsoon</td>
<td>Monsoon</td>
</tr>
<tr>
<td>&lt;1</td>
<td>BSE/n (%)</td>
<td>10/2 (20.0)</td>
<td>9/4 (44.4)</td>
</tr>
<tr>
<td>1-2</td>
<td>BSE/n (%)</td>
<td>22/5 (22.7)</td>
<td>15/5 (33.3)</td>
</tr>
<tr>
<td>&gt;2-4</td>
<td>BSE/n (%)</td>
<td>78/30 (38.5)</td>
<td>29/21 (72.4)</td>
</tr>
<tr>
<td>&gt;4-10</td>
<td>BSE/n (%)</td>
<td>154/54 (35.1)</td>
<td>78/23 (29.5)</td>
</tr>
<tr>
<td>&gt;10-15</td>
<td>BSE/n (%)</td>
<td>64/16 (25.0)</td>
<td>58/7 (12.1)</td>
</tr>
<tr>
<td>&gt;15</td>
<td>BSE/n (%)</td>
<td>457/71 (15.5)</td>
<td>143/11 (7.7)</td>
</tr>
</tbody>
</table>

n, no positive for malaria parasite; BSE, blood smears examined

Table 2

<table>
<thead>
<tr>
<th>Malaria vector</th>
<th>Mean per trap night density (± SE)</th>
<th>t</th>
<th>Mean per trap night density (± SE)</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-monsoon</td>
<td>Monsoon</td>
<td>Pre-monsoon</td>
<td>Monsoon</td>
</tr>
<tr>
<td>An. dirus</td>
<td>0.5 ± 0.2</td>
<td>1.7 ± 0.3</td>
<td>3.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>An. minimus</td>
<td>4.7 ± 0.9</td>
<td>9.5 ± 1.4</td>
<td>2.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.9 ± 1.5</td>
</tr>
<tr>
<td>An. philippinensis/nivipes</td>
<td>17.5 ± 1.9</td>
<td>13.9 ± 2.3</td>
<td>1.30</td>
<td>14.8 ± 2.8</td>
</tr>
<tr>
<td>Total</td>
<td>22.7 ± 2.0</td>
<td>25.1 ± 2.8</td>
<td>0.56</td>
<td>22.2 ± 3.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>p < 0.01

(SVR = 4.3%) and >10 year (SVR = 2.2%) age groups ($\chi^2 = 3.7$; df = 1; $p > 0.05$).

There were 206 malaria cases recorded during the pre-monsoon season against 76 in the monsoon season. The SFR and SVR during the pre-monsoon season (22.7% and 3.6%) were higher than those during the monsoon season (21.4% and 1.5%) although the differences were not statistically significant ($\chi^2 = 0.17$; df = 1; $p > 0.05$ and $\chi^2 = 3.3$; df = 1; $p > 0.05$). The number of males and females tested positive for malaria were 195 and 87, respectively (Fig 4). The males and females in the area were evenly subjected to malaria risk as there was no significant difference between SFR and SVR for the males (23.4% and 3.3%) and those for the females (20.2 and 2.3%) ($\chi^2 = 1.2$; df = 1; $p > 0.05$ and $\chi^2 = 0.77$; df = 1; $p > 0.05$). There were 179 positive cases in 2007 and 103 positive cases in 2008. The SFR has increased from 21.2% in 2007 to 24.3% in 2008 whereas the SVR has decreased from 3.4% to 2.0%. The annual rate of malaria incidence in the region was not found to fluctuate drastically.
Table 3
Susceptibility of *Anopheles minimus* and *Anopheles philippinensis/nivipes* against DDT and deltamethrin in Belonia Subdivision, Tripura, India, 2007-2008.

<table>
<thead>
<tr>
<th>Insecticide (%)</th>
<th><em>Anopheles</em> species</th>
<th>No. exposed (replicates)</th>
<th>No. dead after 24 hours</th>
<th>Mortality (%)</th>
<th>Control mortality (%)</th>
<th>Corrected mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDT (4%)</td>
<td><em>An. minimus</em></td>
<td>30 (3)</td>
<td>28</td>
<td>93.3</td>
<td>6.7</td>
<td>92.9</td>
</tr>
<tr>
<td></td>
<td><em>An. philippinensis/nivipes</em></td>
<td>30 (3)</td>
<td>29</td>
<td>96.7</td>
<td>3.3</td>
<td>96.6</td>
</tr>
<tr>
<td>Deltamethrin (0.05%)</td>
<td><em>An. minimus</em></td>
<td>30 (3)</td>
<td>30</td>
<td>100</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td><em>An. philippinensis/nivipes</em></td>
<td>30 (3)</td>
<td>30</td>
<td>100</td>
<td>0.0</td>
<td>100</td>
</tr>
</tbody>
</table>

as the difference in the annual SFR and SVR during 2007-2008 were not statistically significant ($\chi^2 = 1.1$; df = 1; $p > 0.05$ and $\chi^2 = 1.7$; df = 1; $p > 0.05$).

The percent parasitemia in *P. falciparum* infections was higher during the pre-monsoon season. The mean (± standard error of mean) percent parasitemia in *P. falciparum* positive cases in 2007 was 0.30 (±0.01) during the pre-monsoon season and 0.25 (±0.02) during the monsoon season. The corresponding in *P. vivax* positive were 0.10 (±0.02) and 0.19 (±0.04), respectively. In 2008, the percent parasitemia recorded in *P. falciparum* positive cases was 0.37 (±0.02) and 0.26 (±0.02) during pre-monsoon and monsoon seasons, respectively. The percent parasitemia in *P. vivax* infections during pre-monsoon and monsoon seasons in 2008 were 0.28 (±0.05) and 0.28 (±0.0), respectively. The difference in *P. falciparum* percent parasitemia between the years and also between the seasons were statistically significant ($t = 3.04; p<0.01$ and $t = 3.58; p<0.001$). In the case of *P. vivax* infections, the percent parasitemia was not found to differ significantly between the years or the seasons ($t = 0.83; p > 0.05$ and $t = 1.29; p > 0.05$). Among the 282 cases tested positive for malaria, 15 were detected to have *P. falciparum* - *P. vivax* mixed infection which were grouped under *P. falciparum* positive cases. The mean *P. falciparum* percent parasitemia in the mixed infection cases (0.24 ± 0.02) was significantly higher than the mean *P. vivax* parasitemia (0.06 ± 0.01) ($t = 10.8; df = 14; p<0.001$).

**Entomological studies**

Major malaria vectors recorded in light trap collections were *An. dirus*, *An. minimus* and *An. philippinensis/nivipes* (Table 2). The light trap collections indicated that the mean trap night density (PTN ± SEmean) of *An. dirus* was 0.5 ± 0.2 in 2007 which had increased to 1.7 ± 0.3 in the next year. However, there was no appreciable difference in the density of *An. dirus* during pre-monsoon (1.5 ± 0.4) as compared to monsoon season (0.7 ± 0.2). *An. minimus* mosquito density increased from 4.7 (±0.9) in 2007 to 9.5 (±1.4) in 2008 although the difference between the pre-monsoon (5.9±1.5) and monsoon season (8.3±1.4) densities was not significant.

There was a decline in the density of *An. philippinensis/nivipes* from 17.5 (±1.9) in 2007 to 13.9 (±2.3) in 2008 and from 16.6 (±2.5) in monsoon season to 14.8± 2.8 in pre-monsoon season. The mean trap night density of vector species was 22.7 (±2.0) in 2007 and 25.1 (±2.8) in 2008 and from...
25.6 ± 2.4 during the monsoon season to 22.2 ± 3.1 during the pre-monsoon season.

The mortality rates seen with 4% DDT against *Anopheles minimus* and *Anopheles philippinensis/nivipes* were 92.9%, and 96.6%, respectively, and with 0.05% deltamethrin were 100% for both species (Table 3).

**Drug resistance studies**

Out of the 37 volunteers in whom the chloroquine resistance studies were carried out, early treatment failure (ETF) was observed in 11 and late treatment failure in 2. The occurrence of ETF was highest in children <5 years old (62.5%) followed by the age groups >15 years (25%) and 5-15 years (20%). LTF was seen in 12.5% of children <5 years old, and 4% of children 5-15 years old. There was no LTF observed in persons >15 years old. Adequate clinical and parasitological response (ACPR) was >75% for subjects >5 years old and 25% in subjects <5 years old.

**DISCUSSION**

The present study indicated that the rates of malaria incidence along the Indo-Bangladesh border areas in Tripura varied to a considerable extent between the age groups but not over the years. The percent *falciparum* parasitemia was highest during the pre-monsoon season in comparison with the monsoon season.

*An. dirus*, *An. minimus* and *An. philippinensis/nivipes* were the major malaria vectors observed, which were susceptible to commonly used insecticides. Emergence of chloroquine resistance in the area was noticeable since there was treatment failure in more than one third of the cases.

Malaria transmission is perennial and persistent in northeastern India with peak transmission occurring during May-September (Dev et al, 2003). Monthly data of malaria incidence in the study area indicated a high incidence during June-July. An analysis of fever surveillance data in the neighboring state of Assam also indicated a higher incidence of malaria during June-August. The biting rate of the malaria vector *An. minimus* was found to attain a sharp peak during May-June (Dev et al, 2004).

In the present study, most of the malaria cases reported from the region were attributed to *P. falciparum* (88.3%). More than 60% of the patients were children below 15 years of age and children of the age groups 1-4 were found to be highly susceptible. Dev et al (2004) reported that there was significant difference among the age groups with regard to incidence of malaria in the state of Assam, northeastern India. There was an increasing trend in the proportion of malaria with age in males up to 15 years of age. The prevalence of falciparum malaria among children in Central India was highest in children 4-8 years of age followed by those in 1-4 years age group (Singh et al, 2006) whereas 10-14 year olds were the most susceptible in Gujarat, India (Srivastava and Yadav, 2000).

There was no gender bias in the incidence of malaria although the males were generally more exposed to the vectors due to their outdoor activity and poor clothing. No appreciable difference was observed in the proportion of malaria cases between the sexes in Assam except in 5-15 year olds in whom the proportion of females was lower (Dev et al, 2004). Singh et al (2003) observed equal parasite rates in both sexes in all age groups in Central India.

The percent parasitemia among the village population was found to vary be-
Malaria in Tripura, India

tween the seasons and years. More than 5% of malaria positive cases recorded in the study area were detected to be having *P. falciparum/P. vivax* mixed infections although these are generally considered to be as *P. falciparum* infections and treated accordingly. *P. falciparum* was clearly the dominant parasite species as the percent parasitemia of *P. falciparum* in mixed infections was higher than that of *P. vivax*.

Entomological monitoring through light trap collections indicated the presence of three malaria vectors, namely, *An. dirus*, *An. minimus* and *An. philippinensis/nivipes*. The persistence of malaria in the northeastern India even after the control of *An. minimus* with DDT led to the recognition of *An. dirus* as a major vector of the disease (Prakash *et al.*, 1997). The ‘dirus complex’ consists of at least seven sibling species and is prevalent in forest and forest-fringe areas. *An. baimaiii* is a highly anthropophagic species which was reported to be an efficient vector in the northeastern India (Dutta *et al.*, 1996; Prakash *et al.*, 2006). *An. minimus* is considered as a species complex consisting of three sibling species. *An. minimus s.s.* is highly anthropophagic and is an efficient malaria vector in the northeastern India with an anthropophilic index of 93% (Dev, 1996; Dev *et al.*, 2003). *An. philippinensis* is a vector of malaria in the plains of Bangladesh and hence its role in disease transmission in Tripura need to be investigated further (Prakash *et al.*, 1998).

Insecticide susceptibility studies on malaria vectors indicated more than 90% susceptibility against DDT and 100% against deltamethrin. Indoor residual spraying with 4% DDT has been undertaken in the houses as per the guidelines of National Vector Borne Disease Control Programme (NVBCP). The malaria vectors *An. culicifacies* and *An. stephensi* have been reported to be resistant to DDT in India (Dash *et al.*, 2008) and the emergence of resistance to synthetic pyrethroids in *An. culicifacies* has been recorded (Singh *et al.*, 2002). Continuous monitoring of insecticide susceptibility status in the major malaria vectors has to be an integral part of vector control operations in the region.

Chloroquine is the most frequently used drug for treatment of malaria not only due to its low cost and low toxicity but also due to its effectiveness against all malaria and its relative ease to manufacture (Nuwaha, 2001). A key factor in the resurgence of malaria is the development and spread of drug resistant falciparum malaria which pose great challenges to malaria control (Bloland, 2001). In the present study, treatment failure following chloroquine administration was observed in 35% of the subjects with malaria. In Sonitpur District of Assam, India, 83% of the malaria cases treated with chloroquine were found to be sensitive to the drug (Baruah *et al.*, 2005). Studies conducted in Indo-Myanmar border areas in Arunachal Pradesh State revealed that treatment failure with chloroquine occurred in 83.1% of patients (Mohapatra *et al.*, 2003). Nevertheless, chloroquine remains as the principal drug for malaria treatment in India, except in areas with more than 10% resistance cases (Sharma, 2007).

Studies on malaria epidemiology of such magnitude and extent have been conducted for the first time in this part of the country. These studies indicated that the region continues to have high rates of malaria incidence. Hence, malaria control activities by the local health authorities need to be further strengthened. Since the potential vectors were found susceptible to the DDT there is no need to switch to
other insecticides. High prevalence of chloroquine resistance in the area necessitates the use of artesunate combination therapy.

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