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DDT & deltamethrin resistance status of known Japanese encephalitis vectors in Assam, India

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***Background & objective*: Japanese encephalitis (JE) outbreaks are common in Assam, northeastern State of India. Information on resistance in known JE vectors in the affected area is important for effective control measures. This study was undertaken to determine the species abundance of JE vectors endemic to Sibsagar district of Assam, and their susceptibility against DDT and deltamethrin.**

***Methods*: Adult mosquitoes were collected using CDC light trap and aspirators from human dwellings from 13 endemic villages falling under three Primary Health Centres. Collected mosquitoes were identified and unfed female mosquitoes were used for DDT and deltamethrin sensitivity bioassay. The bioassay was performed following WHO protocol using standard susceptibility test kit. Knockdown time (KDT) was monitored at every 10 minutes intervals, whereas mortalities were recorded 24 h post-exposure. Vector density and resistance status were mapped using geographic information system (GIS) technique.**

***Results*: A total of 7655 mosquitoes were sampled under three genera, *i.e. Anopheles*, *Culex* and *Mansonia*, and nine species, the JE vector *Cx. vishnui* group (31.78%) was the most predominant species, followed by *Ma. uniformis* (16.81%) and *Ma. indiana* (16.45%). All vector species were suspected to be resistant to DDT and sensitive to deltamethrin, except *Ma. indiana*, which was suspected to deltamethrin resistant. The KDT_{50} and KDT_{95} values of vector mosquitoes for DDT were significantly higher as compared to deltamethrin. The probit model used to estimate KDT_{50} and KDT_{95} values did not display normal distribution of percentage knockdown with time for all the vectors tested for DDT and deltamethrin, except for *Ma. indiana* for deltamethrin assay and *Cx. gelidus* for the DDT assay.**

***Interpretation & conclusion:* Differences in insecticide resistance status were observed between insecticides and vector species. The results of this study provided baseline data on insecticide resistance in known JE vectors of Sibsagar, Assam. The maps generated may allow better communication in control operations and comparison of changes in susceptibility status of these vectors over time.**

Key words DDT - deltamethrin - insecticide resistance - JE vector - knock down time - WHO bioassay

In spite of Japanese encephalitis (JE) emerging as a major health problem in southeast Asian countries, the vector abundance and insecticide susceptibility status of known JE vectors has not been monitored regularly. During the last few decades major JE outbreaks have been reported from different parts of India predominantly in the rural areas[1]. The JE related mortality, morbidity and disability adjusted life years (DALYs) are alarming posing serious challenge to the concerted control efforts[2,3]. In the northeastern States of India, the disease appears in sporadic outbreaks with its peak during the rainy season[4,5]. Population-wise Assam is the largest State among the eight States in northeastern India and plays a significant role in the economy of the region[6]. JE outbreaks are common and occur at regular intervals in different parts of India[7]. Dibrugarh is one of the most affected districts of Assam and since 1978 human cases have been regularly reported from this district[8]. In India, of the 16 mosquito species detected harbouring JE virus, *Culex tritaeniorhynchus* has been reported as the prominent JE vector[1,9]. In addition, mosquitoes of *Cx. vishnui* subgroup have been recognized for many years as important vector associated with JE transmission in India[9,10]. Information on the population dynamics of mosquitoes, particularly vectors is necessary for implementation of control measures. In any given area common mosquito species occur during epidemics, however, the abundance of any species depends more upon the availability of preferential feeding host, breeding habitats and survival rates and has direct relevance with the disease transmission.

Studies have shown that use of insecticides has reduced vector density during the disease outbreaks[11]. The JE vectors breeding in agricultural habitats are under tremendous selection pressure of the insecticides sprayed in the fields and, therefore, deserve routine monitoring for their sensitivity status against insecticides used in the control operations[12]. Vector mosquito resistance to the common insecticides has been reported from many Asian countries, including India[12-19]. The level of insecticide resistance has been found to vary even in relatively close areas and during the different seasons[15,20]. These studies suggest that extrapolations of findings from one area to another (even in a small geographical scale) may be inappropriate. Therefore, addressing the insecticide resistance problem among different JE vectors from various endemic areas at micro-level is crucial.

In the present study, investigation was carried out to collect information on the prevalence of known Japanese encephalitis vectors and their resistance/ sensitivity status against DDT and deltamethrin in the study area. DDT is used for indoor residual spray, while deltamethrin is used for the impregnation of bednets in the region, therefore, these two insecticides were chosen for the susceptibility tests.

Material & Methods

Study area: JE vectors were collected from 13 villages falling under three Primary Health Centres (PHCs, Demow, Geleki and Gaurisagar) of Sibsagar district of Assam during peak JE season (July - August) in 2011. The Sibsagar district (94.25° and 95.25° E, 25.45° and 27.15° N) lies on the north bank of river Brahmaputra and has an elevation of 86.6 m (mean sea level). Villages were selected on the basis of the risk of JE transmission for which the inputs from district health staff engaged in the interventions were taken (5 each under Demow and Geleki PHCs and 3 in Gaurisagar PHC). Climate is humid, subtropical and the temperature varies from 8°C in winters to 35°C in the summers. Hot and humid climate, paddy fields, perennial slow flowing streams, irrigation drains/ditches and duck rearing ponds provide bioclimate of choice for abundance of vector mosquitoes.

Adult mosquito sampling and identification: Adult mosquitoes were collected using 6 volts battery operated CDC miniature light trap (John Hock, USA) hung at two meters from the ground level in human dwellings overnight. Hand held mouth aspirators (John Hock, USA) were used to collect the adult mosquitoes resting on the wall, roof, clothes and other surfaces inside the houses. At least four households were selected for the mosquito collection in each of the study village. Smoking by burning hay and other illuminations were prevented during the mosquito collection. Collected mosquitoes were identified upto species level. *Cx. vishnui, Cx. pseudovishnui* and *Cx. tritaeniorhynchus* were clubbed into a group. Preliminary identification of live mosquitoes was carried out morphologically in the glass tubes before conducting the susceptibility assay, which was confirmed after the completion of assay. The unfed female mosquitoes were used for the insecticide sensitivity bioassay so that the tested specimens have physiological status not interfering with the enzymatic activity. The assays were conducted in the batches of 25-35 numbers per test in the field itself. During the

collection period, monsoon season was prevailing in the study area with a temperature and humidity ranging from 25 to 35°C and 60 to 95 per cent, respectively.

WHO insecticide bioassay: The bioassay was performed following standard WHO protocol[21]. DDT (4%), deltamethrin (0.05%) and silicone oil pre-impregnated papers were obtained from vector control research unit, Universiti Sains Malaysia, Malaysia. Mortalities were recorded 24 h post exposure and sensitivity status was graded as per the recommended criteria.

Data analysis and interpretation: Vector density for each PHC (all study sites of each PHC pooled) was expressed in mean ± standard error mean (mean ± SEM) and compared using 1-way analysis of variance (ANOVA), followed by Tukey Kramer test of multiple comparison. Mortality rates recorded were corrected using Schneider-Orelli's formula[22]. Insecticide sensitivity status of mosquitoes was determined as follow[23]. corrected mortality rates <80 per cent indicates resistant; corrected mortality rates >98 per cent indicates fully sensitive; and corrected mortality ranging between >80 to <98 per cent indicates suspected resistance that needs to be verified.

Knockdown rates (KDR) among all vector species for each insecticide were compared using repeated ANOVA. Student's t-test was used to compare the KDR of each vector species for both the insecticides. Similarly, KDR at 10 min intervals for both the insecticides were compared using paired Student's t-test. Knockdown time (KDT_{50} and KDT_{95}) for the mosquito vectors were determined by log-probit method using Log dose probit (Ldp) Line software (Ehabsoft, Egypt). Fitment of probit was assessed using chi-square analysis, whereas the overall significance of the multiple-tests was determined following Bonferroni procedure.

Mapping of vectors and insecticide resistance: Geographical information of the 13 study sites was recorded using global positioning system (Oregon-550, Garmin USA), onto which the densities of nine vector species were marked. DDT and deltamethrin susceptibility of six JE vectors was determined collectively for each PHC. The maps were prepared using Arc GIS version 10 computer programme (Esri, USA).

Results

Vector abundance: Using light traps and aspirators (latter for indoor resting collection), a total of 9650 mosquitoes belonging to five genera, *viz*., *Anopheles* (7 species), *Culex* (6 species), *Mansonia* (3 species), *Coquillettidia* (1 species) and *Armigeres* (1 species) were collected during the study. Of all these, 7655 corresponding to three genera (*Anopheles*, *Culex* and *Mansonia*) and nine species were among the known JE vectors reported in the Indian subcontinent. Prevalence and distribution of JE vectors (mean ± SEM) is shown in Table I. Overall vector density was significantly high in the Gaurisagar PHC as compared to the other two Primary Health Centres (P=0.02). Similarly, the vector density was significantly different among the study villages of each PHC (P<0.0001 for all the three Primary Health Centres). *Cx. vishnui* group (31.78%) was the most predominant species of all collected vector species (all data pooled) followed by *Ma. uniformis* (16.81%)

Table I. Summary of known JE vectors collected in the study area

JE vector	Study area (Primary Health Centres)			*P* value
	Demow	Geleki	Gaurisagar	
Anopheles barbirostris	11 ± 3.0	14.0 ± 4.8	199.7 ± 25.0	0.001
Culex bitaeniorhynchus	4.6 ± 0.6	9.4 ± 3.3	38.0 ± 17.2	0.020
Cx. gelidus	9.6 ± 2.5	6.0 ± 2.9	155.7 ± 30.1	0.001
Cx. quinquefasciatus	3.6 ± 1.5	9.4 ± 1.2	2.3 ± 1.3	0.010
Cx. vishnui group	128.4 ± 7.5	166.6 ± 27.2	319.3 ± 50.8	0.003
Cx. whitmorei	5.2 ± 1.2	14.4 ± 5.3	26.7 ± 7.5	0.040
Mansonia uniformis	17.2 ± 3.6	149.0 ± 34.2	152.0 ± 38.5	0.007
Ma. annulifera	9.4 ± 2.8	39.6 ± 23.9	241.7 ± 41.1	0.001
Ma. indiana	0.8 ± 0.6	184.4 ± 35.9	117.7 ± 12.4	0.001

Values are given as mean ± SEM

Table II. Percentage mortality, KDT_{50} and KDT_{95} values (minutes) of known JE vectors for DDT and deltamethrin

	4% DDT				0.05% deltamethrin			
Vector	% mortality (N)	KDT 50	KDT 95	χ^2	% mortality (N)	KDT 50	KDT 95	χ^2
Ma. uniformis	90.98 (255)	32.67	131.87	20.79	100 (170)	21.57	47.32	20.02
Cx. vishnui group	86.53 (611)	31.60	95.21	160.46	99.69 (640)	16.06	77.66	162.86
Ma. annulifera	96.52 (219)	33.48	90.43	140.27	98.76 (175)	16.39	46.22	15.36
Cx. gelidus	81.4 (112)	47.81	304.97	4.84[#]	100 (130)	12.43	27.26	7.36
Ma. indiana	95.6 (91)	29.60	88.79	31.94	97.1 (72)	11.77	59.43	2.65[#]
An. barbirostris	97.35 (151)	31.97	156.40	22.18	100 (170)	16.92	55.88	9.76

[#] Non significant at 95% confidence interval; KDT, knockdown time

and *Ma. indiana* (16.45%) (P<0.0001). In Demow PHC, *Cx. vishnui* group density was significantly higher than the other two vectors (P<0.0001), whereas in Geleki PHC, the density of these three vectors was similar. In Gaurisagar PHC, unlike the other two Primary Health Centres, *An. barbirostris* and *Ma. annulifera* were widely distributed and their densities were similar to *Cx. vishnui* group mosquito (Table I).

WHO insecticide bioassay: The results of susceptibility status of six potential JE vectors to the diagnostic dosages of DDT and deltamethrin are shown in Table II. The mortality, after 24 h recovery period was <98 per cent for DDT in all the tested vector mosquitoes, which shows that resistance is suspected in the vector population. The highest mortality to DDT (97.35%) was recorded in *An. barbirostris*, whereas lowest (81.4%) was recorded in *Cx. gelidus* mosquito. On the other hand, mortality after 24 h recovery was >98 per cent for deltamethrin in the vector mosquitoes, except in *Ma. indiana*, whereas only 97.1 per cent mortality could be achieved. For DDT, KDT_{50} and KDT_{95} values (47.81 and 304.97 min, respectively) were highest in *Cx. gelidus*, whereas lowest (29.60 and 88.79 min respectively) in *Ma. indiana*. For deltamethrin, highest 50 and 95% knockdown values were recorded in *Ma. uniformis* (21.57 min) and *Cx. vishnui* group (77.66 min), respectively, and lowest were found in *Cx. vishnui* group (16.06 min) and *Cx. gelidus* (27.26 min) mosquitoes, respectively. The KDT_{50} and KDT_{95} values of vector mosquitoes (data pooled) for deltamethrin was significantly less as compared to the DDT (P<0.00 for KDT_{50} and P=0.03; for KDT_{95}). Similarly, the knockdown rates at 10 min intervals were significantly different between both the insecticides (P=0.02). Probit model used to estimate KDT_{50} and KDT_{95} values did not display normal distribution of percentage knockdown with time for all the vectors tested for DDT and deltamethrin, except for *Ma. indiana* for deltamethrin assay and *Cx. gelidus* for the DDT assay. The KDT_{50} and KDT_{95} values of DDT for all the vector mosquitoes were increased by factors ranging 1.5-3.8 (in KDT_{50}) and 1.2-11.2 (in KDT_{95}) as compared to deltamethrin.

Vector density and insecticide resistance mapping: Mosquito density of JE vectors was determined using pie method in all the study sites. The density of each vector has been shown using different colours. To depict the resistance status of deltamethrin and DDT for different vectors, the vector density of study sites were aggregated at PHC level and the resistance status was shown using green colour for sensitive and yellow colour for suspected resistance in the vector mosquitoes (Fig.). The map shows that all the vector species tested, except *Ma. indiana*, were sensitive to deltamethrin, whereas *Ma. indiana* showed suspected resistance status. On the other hand, all the species showed suspected resistance status for DDT.

Discussion

The present study was undertaken in three most affected Primary Health Centres of Sibsagar district of Assam. Of the nine known JE vectors, *Cx. bitaeniorhynchus*, *Cx. quinquefasciatus* and *Cx. whitmorei* were collected in very low number, throughout the study period, and the remaining six vectors were widely distributed and collected in large number. *Cx. vishnui* group was most abundant species among all the three Primary Health Centres. The known Indian JE vector density was high in Gaurisagar as compared to the other two Primary Health Centres. The population dynamics of JE vectors largely depends upon rice cultivation, water bodies, temperature and humidity in the rural areas[24]. The Gaurisagar area has many water bodies, duck rearing ponds and large area under paddy cultivation adjacent to the domiciliary

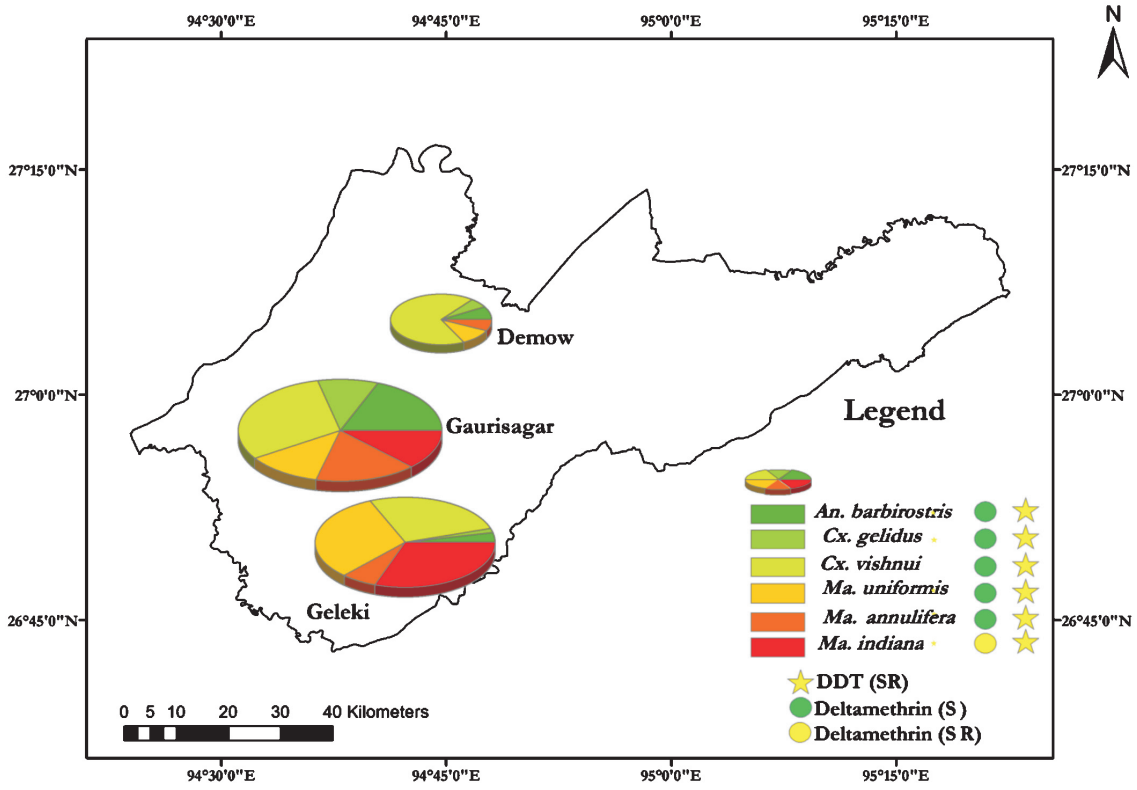

Fig. Density and resistance status of major known JE vectors at PHC level. SR, suspected resistance; S, sensitive.

surroundings. As a result, the JE vector mosquito density was comparatively high in the Gaurisagar. *Cx. vishnui* and *Cx. gelidus* have been reported to be most prevalent JE vector in many parts of Assam[4]. In many parts of India and Southeast Asian countries, *Cx. tritaeniorhynchus* has been recorded in abundance and incriminated as major vector JE vector[1]. Population density of *Cx. vishnui* group was substantially high among all the sampling villages during peak JE period, this species is most likely to be a potential JE vector in the area.

In the study area, DDT is used for indoor residual spray regularly and deltamethrin-treated bednets were recently provided free of cost in limited numbers to the villagers. However, during the study only 80-90 per cent of households were found using treated bednets and many of them were not regularly re-treating their bednets. The results obtained in the present study showed that all the six known JE vector mosquitoes were not completely susceptible to DDT. The mortality obtained at 24 h post-exposure of DDT was >80 per cent and <98 per cent, suggesting further verification data are required in the study area. Similar results have been obtained in the other parts of northeast India, where DDT was tested on *Anopheles* mosquito[14,15]. In all the PHCs studied, the tested mosquitoes were fully susceptible to deltamethrin, except *Ma. indiana*, for which verification data are needed. The high KDT_{50} and KDT_{95} values of DDT as compared to deltamethrin compare well with the fact that DDT has been in use for many years, which have led to the development of resistance among the mosquito species in various parts of Assam[15,25]. Further, the agriculture expansion is also a major factor contributing to resistance development in vectors in the region[4,14]. The KDT_{50} values of deltamethrin observed in the present study were higher than observed elsewhere[15]. The knockdown values are largely associated with the level of use of an insecticide, which would lead to possible resistance in the mosquito species[20]. It was interesting to note that in *Ma. indiana*, the KDT_{50} and KDT_{95} values of DDT were lower than other mosquitoes, suggesting that these were more knockdown sensitive to DDT. The knockdown resistance mechanism is one of the most important forms of resistance, which occurs when insecticide detoxifying enzyme level is elevated in the mosquito[15,26]. The insecticide resistance is a complex

mechanism and depends upon different physiological conditions of insect[25]. It has been shown that use of different insecticides may accelerate cross-resistance among insects[27].

GIS mapping has been used before to find out the malaria hot spots at health subcentre level[28,29]. The maps generated in the present study may be effective in communicating the main findings with the local health authority at implementation level and planning an effective vector control strategy.

In conclusion, the present study confirmed the development of low level DDT resistance in all known JE vectors prevalent in the study area, therefore, control programmes employing DDT as residual insecticide may not achieve satisfactory results. Deltamethrin used in impregnation of bednets could be useful at individual household level. More areas are needed to be scanned and mapped for the insecticide resistance status of major JE vectors.

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