

Entomological investigations into an epidemic of Japanese encephalitis (JE) in northern districts of West Bengal, India (2011-2012)

T. Mariappan, P. Philip Samuel, V. Thenmozhi, R. Paramasivan, Puran Kumar Sharma*, Asit Kumar Biswas* & B.K. Tyagi

*Centre for Research in Medical Entomology (ICMR), Madurai & *West Bengal Public Health & Administrative Service, Darjeeling, India*

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Background & objectives: Japanese encephalitis (JE) is one of the most important arboviral diseases of human beings with outbreaks in many parts of Southeast Asia including India. We present the entomological findings of an outbreak occurred in northern part of West Bengal during 2011-2012 with special emphasis on the role of JE vectors in different seasons.

Methods: Adult mosquito collections were made with the help of mouth aspirators, aided by flash lights during day time resting inside human and animal habitations as indoor, and resting outside field grasses, bushes, underneath of culverts and bridges as outdoor, and in and around the pig enclosures and cattle sheds during dusk period in JE affected villages from Cooch Behar, Dakshin Dinajpur, Darjeeling and Jalpaiguri districts in North West Bengal. In all study villages, a long handled with enamel bowl dipper was used to obtain immature stages of mosquitoes from various breeding habitats.

Results: A total of 19 different types of mosquito breeding habitats were examined for vectors of JE. From these habitats, 23.7 per cent were positive for breeding during the study period. Overall, nine different species were recorded through emergence, but none was positive for JE virus when subjected for detection of virus. Adult mosquitoes of more than 50 per cent of the potential JE vector species obtained through dusk and the rest through indoor and outdoor collections in all seasons. Altogether, 27 different species were recorded. Most of these were JE vectors.

Interpretation & conclusions: Our results showed that in addition to *Cx. vishnui* subgroup, detection of JE virus antigen in *Cx. quinquefasciatus* indicated the possible maintenance of JE virus in nature through poor vector mosquitoes throughout the year. Since, all potential vector species reported elsewhere in India were also found in this region and fluctuated in density in different seasons, a proper integrated vector control programme needs to be implemented to control JE transmission.

Key words *Culex pseudovishnui* - *Cx. quinquefasciatus* - *C. tritaeniorhynchus* - *Cx. vishnui* - Japanese encephalitis - mosquito emergence - vector

Japanese encephalitis (JE) is a mosquito-borne arboviral disease and is the leading cause of morbidity and mortality in South East Asia. The outcome of JE can be fatal in 25 per cent of cases, and it may also result in residual sequelae in 30 to 60 per cent of cases^{1,2}. In India, the disease was first reported in 1955 and subsequently many epidemics have occurred in different parts of the country³⁻⁷. JE virus (JEV) infects a large number of susceptible individuals but only a few develop overt manifestations of the disease⁸. JE is principally a disease of rural agricultural areas, where vector mosquitoes proliferate in close association with pigs, which are the principal vertebrate amplifying hosts. Large aquatic birds may sub serve this function in areas where pigs are absent. Humans are incidental to the transmission cycle^{9,10}. The seasonal incidence of JE varies in different parts of India. In West Bengal, the disease occurred between May and October, and was shown to be related to the summer monsoon. In Assam and Uttar Pradesh epidemics have occurred between September and December¹¹. During 2011-2012, JE outbreaks occurred in many parts of north eastern States including Bihar and North West Bengal. During the recent outbreak JE cases were reported from four different districts (*i.e.* Cooch Behar, Dakshin Dinajpur, Darjeeling and Jalpaiguri) of North West Bengal from the first quarter of 2011 onwards. After confirmation of JE infection, an entomological investigation was initiated by the team of Centre for Research in Medical Entomology (CRME), Madurai, Tamil Nadu, India, in September 2011 onwards to complete a year round study at the quarterly interval to pin point the role of different species of mosquito vector. The results of this entomological investigation are presented here.

Material & Methods

Study area: After the JE cases reported in the four districts of Northern West Bengal, four surveys were carried out at the quarterly intervals (*i.e.* September 15, 2011 to October 2, 2011 in 19 villages, January 18, 2012 to February 6, 2012 in 19 villages, May 10, 2012 to June 3, 2012 in 24 villages and September 11, 2012 to October 10, 2012 in 24 villages) in the four districts.

As per the climatological particulars provided by the Government of West Bengal Office of the Additional Director of Agriculture, North Bengal Region, Jalpaiguri, the JE affected four different regions have five distinct seasons (summer - April to May, monsoons - June to September & October to November, autumn - December to January middle,

winter - January middle to February middle, and spring - February to March). The average rain fall (424.8 mm) occurs in June only. The relative humidity ranges between 60.5 per cent in March and 84.6 per cent in October. The annual mean maximum temperature is 14.9 °C while the mean minimum temperature is 8.9 °C with monthly mean temperatures ranging from 5-17 °C. The average annual precipitation is 309.2 cm, with an average of 126 days of rain in a year. The highest rainfall occurs in July.

Study design: The four districts made a single study unit. Of the four districts, a total of 42 villages were selected (as per the line list submitted by the North West Bengal Medical College Hospitals, Department of Health, West Bengal). Of these 42 villages, four visits were made in nine villages, three visits in seven, two in eight and 13 single visits and the remaining five were not attended due to logistic reasons. Overall, 86 collections of immature stages from various breeding habitats and different types of adult mosquitoes collections from these 37 villages were made.

The criteria followed included minimum three villages from respective districts to study at the quarterly interval. However, nine villages were covered from three districts as designed only, three villages could not be studied from Dakshin Dinajpur during the second visit thereby only three visits made in those three villages (*i.e.* first, third and fourth visits). The remaining villages were covered either single or 2 or 3 visits only as stated above. Most of the sites were under paddy cultivation plus other geographical variations with different altitudes (ranged between 66 and 1500 feet) located behind riverside, tea garden cultivation, adjacent to forested, elevated hilly tracts, very close to big water stored ponds, *etc.* These study sites were chosen on the basis of line list of JE confirmed cases.

Adult mosquitoes collection: In every study village, the various types of mosquito collections undertaken included indoor, outdoor and dusk for adult mosquitoes at the quarterly interval from September 2011 to October 2012. In respective study sites, during day time (*i.e.* 0900 and 1000 h) mosquito catch was made from inside houses including sitting room, bed room, store room and kitchen with the help of mouth aspirators aided by torch lights. Similar collections of resting mosquitoes made from outdoor resting habitats consisted of field grasses, bushes, fencing of open area, underneath of culverts, *etc.* Dusk mosquito collections (from 1915 to 2015 and 1750 to 1850 h during summer and winter, respectively) were made with the help of

mouth aspirators, aided by torch lights in and around pig enclosures and cattle sheds. In identified study villages, per man hour density (PMH) was monitored for each and every types of collections known as indoor, outdoor and dusk. Both blood engorged and unfed adult mosquitoes were subjected for virus isolation¹²⁻¹⁵.

Collections of immature stages: Study villages, various types of breeding habitats were examined for mosquito immature stages by spending not less than an hour with dipper (*i.e.* an enamel bowl with 12.5 cm in diameter fitted with one meter long handle of aluminium rod). The required dips were obtained depending upon the size of various habitats like pits, pools and paddy fields, *etc.* In drain or canal sequential sampling was done at the gap of 2 meters on either side¹⁴. Immature stages of late fourth instar and pupae obtained were kept for emergence to identify the species.

Screening mosquitoes for JE virus: Mosquitoes obtained by different methods were identified upto species level and made into pools in the transit laboratory. The pools were kept in liquid nitrogen and brought to the central laboratory, CRME, Madurai, to examine for virus detection by antigen capture ELISA method employing JE virus specific monoclonal antibody¹⁵.

Statistical analysis: In the present study, approximately eight per cent houses were selected as sample from the sample frame. The appropriate statistical measures were applied along with graphical presentation representing seasonal comparison between various genera of mosquitoes. Per man hour density of mosquitoes were calculated for adult mosquitoes collections.

Results

Immature stages of mosquitoes: In all study sites, a total of 19 different types of mosquito breeding habitats were examined for breeding status (Table I). Of these 19 habitats, 23.7 per cent were found positive for breeding of mosquitoes. The positive breeding habitats in September-October 2011 were 23.2 per cent, 19.8 per cent in January-February 2012, 27.3 per cent in May-June 2012 and 24.9 per cent in September-October 2012. All emerged mosquitoes included *Culex quinquefasciatus*, *Cx. tritaeniorhynchus*, *Cx. gelidus*, *Cx. vishnui*, *Cx. bitaeniorhynchus*, *Cx. fuscocephalus*, *Anopheles peditaeniatus*, *An. barbirostris*, and *Armigeres subalbatus*. All emerged mosquitoes were subjected for virus isolation, but none of these emerged mosquitoes were positive for virus in the laboratory.

Adult mosquito collections: The adult mosquitoes obtained through various collections are shown in Table

II. Indoor resting mosquitoes comprised 20 species belonging to six genera. *Culex* was the predominant genera followed by *Armigeres*, *Anopheles*, *Mansonia*, *Aedes* and *Metalutzia*.

Among *Culex* mosquitoes, *Cx. quinquefasciatus* (57.54%) was predominant followed by *Cx. tritaeniorhynchus* (7.88%), *Cx. gelidus* (4.46%), *Cx. vishnui* (3.18%), *Cx. bitaeniorhynchus* (0.56%) and *Cx. pseudovishnui* (0.16%) and *Cx. infula* (0.08%). Of the five *Anopheles* species, *An. vagus* (7.2%) was predominant followed by *An. subpictus* (5.25%), *An. barbirostris* (0.76%), *An. pallidus* (0.44%) and *An. peditaeniatus* (0.08%). Within two *Mansonia* species, *Ma. uniformis* (1.63%) and *Ma. annulifera* (0.4%) were recorded. The remaining six different species of non-JE vectors namely *Ar. subalbatus* (9.27%), *Ae. aegypti* (1.35%), *Lutzia (Metalutzia) vorax* (0.08%), *Ae. scutellaris* (0.2%), *Ae. albopictus* (0.44%) and *Ae. vexans vexans* (0.08%) were recorded. In all study areas, *Cx. quinquefasciatus* obtained from indoor in all seasons were compared with conventional JE vectors (Fig.). The percentages of *Cx. quinquefasciatus* density obtained in descending order were 95.6 per cent in January-February 2012 (cold months), 83.4 per cent in May-June 2012 (summer months) and 44.3 per cent in September-October 2011 (rainy months) and 10.4 per cent in September-October 2012.

Outdoor resting mosquitoes obtained from the study areas comprised 12 species belonging to four genera. *Culex* was predominant genera followed by *Armigeres*, *Anopheles*, and *Mansonia*. Among *Culex* mosquitoes *Cx. quinquefasciatus* (35.55%) was predominant followed by *Cx. pseudovishnui* (6.25%), *Cx. tritaeniorhynchus* (5.18%), *Cx. gelidus* (4.75%), *Cx. bitaeniorhynchus* (3.13%) and *Cx. vishnui* (1.38%). *Anopheles vagus* (16.38%) was predominant followed by *An. subpictus* (3.43%) and *An. pallidus* (0.38%). In the case of *Mansonia*, *Ma. annulifera* (1.35%) and *Ma. uniformis* (0.4%) were obtained. The remaining non-JE vector *Ar. subalbatus* was also recorded (21.83%). Both indoor as well as outdoor obtained conventional vectors of all *Culex* species were compared respectively as shown in the Figure. The percentage of *Cx. quinquefasciatus* density obtained was 67.3 per cent in January-February 2012 (cold months) 43.3 per cent in September-October 2011 (rainy months), and the collection was not done in the remaining two seasons. In the case of conventional *Anopheles* species, the peak was during rainy months followed by cold period and

Table I. Number of breeding habitats positive for mosquitoes and their significance from different districts of North West Bengal

Habitats	September-October 2011		January-February 2012		May-Jun 2012		September-October 2012		Grand total		%
	Number surveyed	+ve	Number surveyed	+ve	Number surveyed	+ve	Number surveyed	+ve	Number surveyed	+ve	
Cess pool	51	24	6	3	1	0	9	2	67	29	43.3
Cement cistern	0	0	106	1	18	10	42	10	166	21	12.7
Cess pit	25	7	7	5	2	2	0	0	34	14	41.2
Cement tank	10	4	79	7	0	0	0	0	89	11	12.4
Culvert	15	4	0	0	0	0	1	1	16	5	31.3
Drain	18	3	39	16	68	24	16	5	141	48	34.0
Fallow field	0	0	5	3	10	7	6	3	21	13	61.9
Hoof print	2	2	0	0	0	0	0	0	2	2	100.0
Irrigation channel	30	5	3	1	10	0	12	2	55	8	14.5
Manure pit	0	0	0	0	7	2	7	2	14	4	28.6
Mud pool	0	0	0	0	24	9	0	0	24	9	37.5
Paddy field	49	9	13	0	25	8	64	11	151	28	18.5
Pond	25	9	11	8	67	13	40	14	143	44	30.8
River bed pool	12	1	0	0	30	0	0	0	42	1	2.4
Rain water pool	10	0	21	3	1	0	21	6	53	9	17.0
Slow flow stream	1	0	10	4	10	0	0	0	21	4	19.0
Spillage/seepage	1	0	0	0	5	4	0	0	6	4	66.7
Spring slow flowing	1	0	0	0	17	2	6	0	24	2	8.3
Unused well	56	3	14	11	13	3	5	1	88	18	20.5
Total	306	71	314	62	308	84	229	57	1157	274	23.7

Mansonia species was recorded in very low numbers compared with other conventional vector mosquitoes.

Based on dusk collection, a total of 24 different mosquito species were obtained. Those species include 10 *Culex* (*i.e.* 80.39%) which was predominant followed by seven *Anopheles* (5.42%), two *Mansonia* (4.75%), four *Aedes* (0.54%), and one *Armigeres* (8.9%) (Table II). Among *Culex* mosquitoes, *Cx. gelidus* (39.36%) was predominant followed by *Cx. tritaeniorhynchus* (24.32%), *Cx. quinquefasciatus* (14.87%), *Cx. vishnui* (0.86%), *Cx. bitaeniorhynchus* (0.29%), *Cx. fuscocephalus* (0.29%), *Cx. pseudovishnui* (0.15%), *Cx. whitmorei* (0.13%), *Cx. infula* (0.11%)

and *Cx. nilgircus* (0.01). Of the seven *Anopheles* species, *An. vagus* (1.62%) was predominant followed by *An. subpictus* (1.6%), *An. pallidus* (0.96%), *An. barbirostris* (0.62%), *An. peditaeniatus* (0.58%), *An. tessellatus* (0.03%), and *An. stephensi* (0.01%). Among *Mansonia* species, *Ma. annulifera* was 2.62% followed by *Ma. uniformis* (2.13%) The remaining species included *Ar. subalbatus* (8.9%), *Ae. vexans vexans* (0.18%), *Ae. lineatopennis* (0.17%), *Ae. albopictus* (0.15%) and *Ae. pallidostriatus* (0.05%). In dusk collection, the percentage of *Cx. quinquefasciatus* density was 81.4 per cent in January-February 2012 (cold months) 61.9 per cent in May-June 2012, 6.2

Table II. Mosquitoes obtained through indoor (I), Outdoor (O) and dusk (D) in % from four districts in four different periods

Species	Cooch Behar			Dakshin Dinajpur			Darjilling			Jalpaiguri		
	I	O	D	I	O	D	I	O	D	I	O	D
<i>Culex tritaeniorhynchus</i>	21.1	10.4	37.6	19.6	2.7	30.9	1.5	7.6	6.2	1.8	0	14.9
<i>Cx. vishnui</i>	0.5	0.0	1.0	14.5	5.5	1.2	0.0	0.0	0.2	1.4	0	0.6
<i>Cx. pseudovishnui</i>	0.0	0.0	0.0	0.6	0.0	0.2	0.0	0.0	0.0	0.1	25	0.4
<i>Cx. gelidus</i>	5.9	3.7	13.8	11.1	1.8	57.5	0.0	1.0	23.8	5.2	12.5	30.4
<i>Cx. bitaeniorhynchus</i>	0.0	0.0	0.0	1.9	0.0	0.3	0.0	0.0	0.1	0.7	12.5	0.6
<i>Cx. quinquefasciatus</i>	48.1	38.1	19.0	16.0	23.6	2.9	79.7	30.5	38.4	60.5	50	18.4
<i>Cx. fuscocephalus</i>	0.0	0.0	0.1	0.0	0.0	0.4	0.0	0.0	0.4	0.0	0.0	0.0
<i>Cx. whitmorei</i>	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
<i>Cx. infula</i>	0.0	0.0	0.1	0.4	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.2
<i>Cx. nilgircus</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
<i>Mansonia uniformis</i>	0.8	0.7	4.1	1.3	0.9	1.3	0.0	0.0	1.3	1.2	0	3.3
<i>Ma. annulifera</i>	0.5	4.5	2.6	0.9	0.9	0.5	0.0	0.0	0.9	0.5	0	9.5
<i>Armigeres subalbatus</i>	16.0	28.4	11.9	9.2	0.0	0.7	12.2	58.9	22.7	7.5	0	13.4
<i>Anopheles subpictus</i>	2.1	12.7	2.7	9.0	0.0	1.4	1.0	1.0	1.4	4.5	0	1.4
<i>An. stephensi</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>An. barbirostris</i>	2.9	0.0	0.8	1.7	0.0	0.7	0.0	0.0	0.2	0.0	0.0	0.7
<i>An. vagus</i>	0.8	0.0	0.8	13.9	64.5	1.6	2.6	1.0	1.9	12.2	0.0	2.1
<i>An. peditaeniatus</i>	0.0	0.0	0.7	0.0	0.0	0.3	0.1	0.0	0.4	0.0	0.0	1.5
<i>An. pallidus</i>	0.0	1.5	3.2	0.0	0.0	0.0	1.1	0.0	1.5	0.1	0.0	1.0
<i>An. tessellatus</i>	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
<i>Aedes albopictus</i>	0.5	0.0	0.2	0.0	0.0	0.1	1.0	0.0	0.2	0.0	0.0	0.4
<i>Ae. lineatopennis</i>	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6
<i>Ae. aegypti</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	4.1	0.0	0.0
<i>Ae. vexans vexans</i>	0.3	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0
<i>Ae. scutellaris</i>	0.3	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0
<i>Lutzia (Metalutzia) vorax</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
<i>Ae. pallidostratus</i>	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

per cent in September-October 2011 (rainy months), and 1.3 per cent in September-October 2012 (rainy months).

The percentage of *Cx. quinquefasciatus* contribution was low compared with conventional *Culex* mosquitoes obtained during rainy months where more habitats supported conventional *Culex* species. Other than *Cx. quinquefasciatus*, two peaks were noticed by the contribution of major vectors of JE, *Cx. tritaeniorhynchus* followed by *Cx. vishnui*, *Cx. bitaeniorhynchus*, *Cx. fuscocephalus*, *Cx.*

pseudovishnui, *Cx. whitmorei*, *Cx. infula* and *Cx. nilgircus*. Per man hour (PMH) densities of mosquitoes in the four different periods covering all four districts resulted from September-October 2011 to September-October 2012 were 85.13, 19.77, 34.45 and 129.25 PMH. The highest density recorded in rainy months indicated very high risk of transmission of JE during that particular period.

Detection of JE virus in mosquitoes: All mosquitoes were subjected to viral assay and found positive for JE virus which in a descending order with positive pools

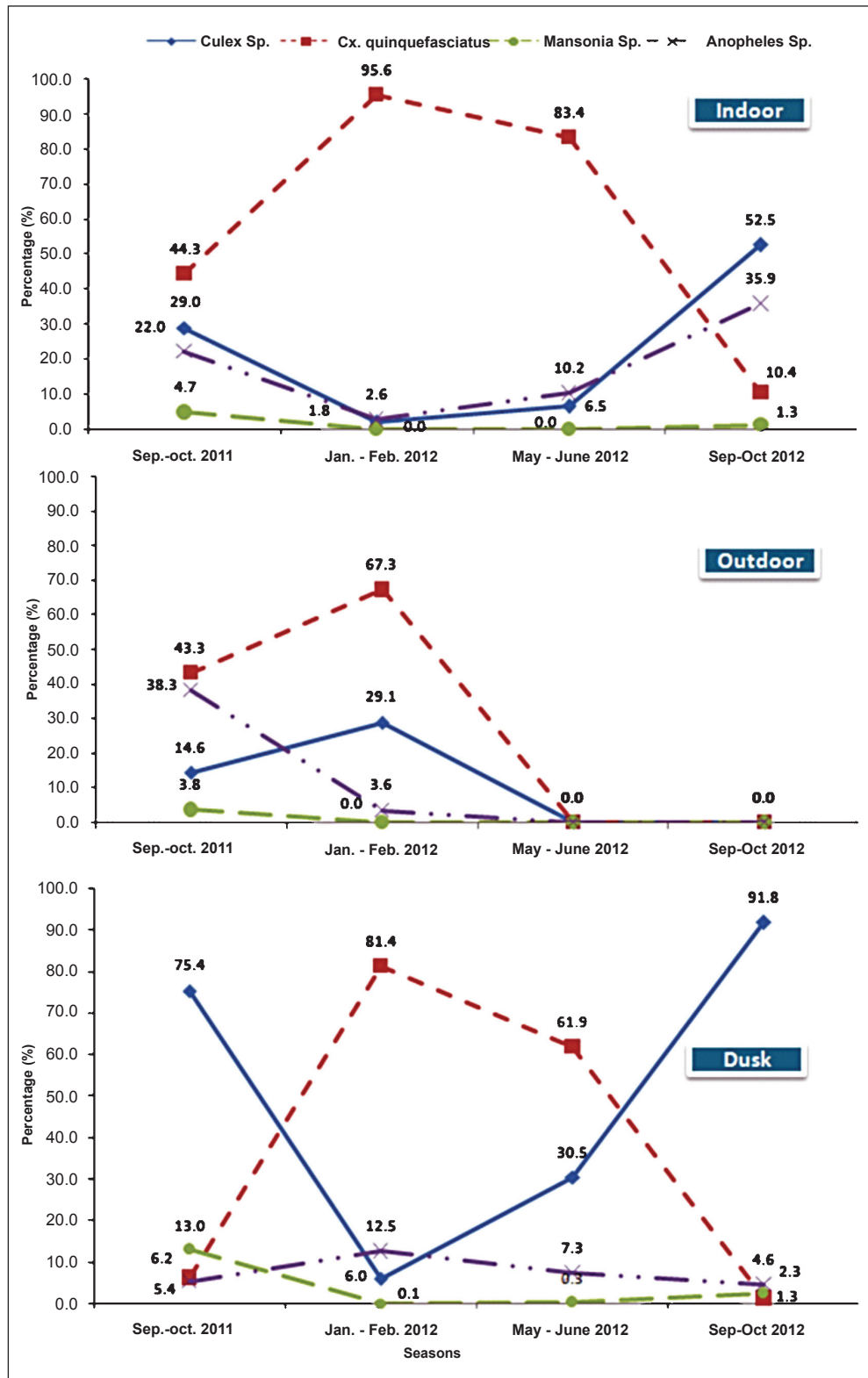


Fig. Conventional JE vectors compared with *Cx. quinquefasciatus* obtained through indoor, outdoor and dusk collections

in parentheses were as follows: *Cx. tritaeniorhynchus* (3), *Cx. gelidus* (3), *Cx. quinquefasciatus* (2), *Cx. pseudovishnui* (2) *Ma. uniformis* (2), *Cx. vishnui* (1) *Cx. fuscocephalus* (1) *Ma. annulifera* (1). A total of 15 pools were found positive for JE virus antigen during the entire study period from eight different species. Most of the pools found positive for JE virus in rainy months proved the active virus circulation in this area and indicated a high risk of transmission due to the major vectors in this region.

Discussion

In the four districts surveyed in North West Bengal, the adult mosquitoes collections indicated that the various habitats were contributing vectors throughout the year. More than 50 per cent of the potential JE vector species obtained through dusk collection and the remaining through other collections pointed out that people in this area were at a risk for transmission of JE. Most of the species were potential vectors and very few were non-vector mosquitoes representing genera *Anopheles*, *Aedes* and *Armigeres*. Therefore, the area was prone for outbreak situation of JE in different seasons with a relatively high risk during rainy months.

JE virus is transmitted naturally between ardeid birds and pigs by mosquito species belonging to the *Cx. vishnui* subgroup, comprising *Cx. tritaeniorhynchus*, *Cx. vishnui* and *Cx. pseudovishnui* in India¹⁶. The present epidemic in the districts of North West Bengal confirmed the role of *Cx. vishnui* subgroup in virus transmission. The significant detection of JE virus antigen in *Cx. quinquefasciatus* indicated the possible maintenance of JE virus in nature through poor vector mosquitoes throughout the year. Systematic studies are needed to prove the role of *Cx. quinquefasciatus* in maintenance/transmission of JE virus with molecular tools. It has been reported that *Cx. quinquefasciatus* is the most common domestic species in urban, semi urban and rural areas and strongly anthropophilic, and JE virus isolation has been made from Kolar district, Karnataka¹⁷⁻¹⁹. Though JE is a vaccine preventable disease²⁰, the transmission can be reduced drastically by exercising vector control measures in JE endemic areas. In paddy fields, composite fish culture for controlling mosquitoes is being practiced in many parts of our country²¹ needs to be exercised in this region also. In Bellary district of Karnataka *Cx. tritaeniorhynchus* was found to be resting indoors in large numbers during JE transmission and house spray was suggested with residual insecticide to suppress the adult population²².

However, in the JE affected belt in North West Bengal, the densities of the *Culex* subgenera species were found throughout the year increasing the chances of transmission. Since epidemics of JE continue to occur in different parts of the country and the diseases is spreading to newer areas, in-depth intensive studies need to be undertaken to highlight the role of different vector species towards JE transmission and to devise appropriate feasible control measures.

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References

1. Tobias E. Erlanger, Svenja Weiss, Jennifer Keiser, Jürg Utzinger, Karin Wiedenmayer. Past, Present, and Future of Japanese Encephalitis. *Emerg Infect Dis* 2009; 15 : 1-7.
2. Grant L Campbell, Susan L Hills, Marc Fischer, Julie A Jacobson, Charles H Hoke, Joachim M Hombach, *et al.* Estimated global incidence of Japanese encephalitis: a systematic review. *Bull World Health Organ* 2011; 89 : 766-74.
3. Gajanan A. Epidemiology and surveillance of Japanese encephalitis in Tamil Nadu. *ICMR Bull* 1998; 28 : 33-7.
4. Kanojia PC, Shetty PS, Geevarghese G. A long-term study on vector abundance & seasonal prevalence in relation to the occurrence of Japanese encephalitis in Gorakhpur district, Uttar Pradesh. *Indian J Med Res* 2003; 117 : 104-10.
5. US, Rao MS, Arunachalam N. The effects of climatic factors on the distribution and abundance of Japanese encephalitis vectors in Kurnool district of Andhra Pradesh, India. *J Vector Borne Dis* 2010; 47 : 26-32.
6. Mourya DT, Ilkal MA, Mishra AC, Jacob PG, Pant U, Ramanujam S, *et al.* Isolation of Japanese encephalitis virus from mosquitoes collected in Karnataka state, India from 1985 to 1987. *Trans R Soc Trop Med Hyg* 1989; 83 : 550-2.
7. Mishra AC, Jacob PG, Ramanujam S, Bhat HR, Pavri KM. Mosquito vectors of Japanese encephalitis epidemic (1983) in Mandya district (India). *Indian J Med Res* 1984; 80 : 377-89.
8. George S, Yergolkar PN, Kamala, Kamala CS. Outbreak of encephalitis in Bellary district of Karnataka & adjoining areas of Andhra Pradesh. *Indian J Med Res* 1990; 91 : 328-30.
9. Chakravarty SK, Sarkar JK, Chakravarty MS, Mukherjee MK, Mukherjee KK. The first epidemic of Japanese encephalitis studied in India-virological studied. *Indian J Med Res* 1975; 63 : 77-82.

10. Rajagopalan PK, Panicker KN. A note on the 1976 epidemic of Japanese encephalitis in Burdwan district, West Bengal. *Indian J Med Res* 1978; 68 : 3938.
11. Reuben R, Gajanana A. Japanese encephalitis in India. *Indian J Pediatr* 1997; 64 : 243-51.
12. Mitchell CJ, Chen PS. Ecological studies on the mosquito vectors of Japanese encephalitis. *Bull World Health Organ* 1973; 49 : 287-92.
13. Geevarghese G, Mishra AC, Jacob PG, Bhat HR. Studies on the mosquito vectors of Japanese encephalitis virus in Mandya district, Karnataka, India. *Southeast Asian J Trop Med Public Health* 1994; 25 : 378-82.
14. Self LS, Shin HK, Kim KH, Lee KW, Chow CY, Hong HK. Ecological studies on *Culex tritaeniorhynchus* as a vector of Japanese encephalitis. *Bull. World Health Organ* 1973; 49 : 41-7.
15. Gajanana A, Rajendran R, Thenmozhi V, Samuel P, Tsai TF, Reuben R. Comparative evaluation of bioassay and ELISA for detection of Japanese encephalitis virus in field collected mosquitoes. *Southeast Asian J Trop Med Public Health* 1995; 26 : 91-7.
16. Reuben R, Thenmozhi V, Samuel PP, Gajanana A, Mani TR, Mosquito blood feeding patterns as a factor in the epidemiology of Japanese encephalitis in southern India. *Am J Trop Med Hyg* 1992; 46 : 654-63.
17. Banerjee K, Deshmukh PK, Ilkal MA, Dhanda V. Experimental transmission of Japanese encephalitis virus through *Anopheles tessellatus* and *Culex fatigans* mosquitoes. *Indian J Med Res* 1977; 65 : 746-52.
18. Mourya DT, Ilkal MA, Mishra AC, Jacob PG, Pant U, Ramanujam S, *et al.* Isolation of Japanese encephalitis virus from mosquitoes collected in Karnataka state, India from 1985 to 1987. *Trans R Soc Trop Med Hyg* 1989; 83 : 550-2.
19. Banerjee K, Mahadev PV, Ilkal MA, Mishra AC, Dhanda V, Modi GB, *et al.* Isolation of Japanese encephalitis virus from mosquitoes collected in Bankura district, West Bengal during October 1974 to December 1975. *Indian J Med Res* 1979; 69 : 201-5.
20. Solomon T, Kneen R, Dung NM, Khanh VC, Thuy TT, Ha DQ, *et al.* Poliomyelitis-like illness due to Japanese encephalitis virus. *Lancet* 1998; 351 : 1094-7.
21. Victor TJ, Chandrasekaran B, Reuben R. Composite fish culture for mosquito control in rice fields in southern India. *Southeast Asian J Trop Med Public Health* 1994; 25 : 522-7.
22. Kanojia PC. Ecological study on mosquito vectors of Japanese encephalitis virus in Bellary district, Karnataka. *Indian J Med Res* 2007; 126 : 152-7.

Reprint requests: Dr B.K. Tyagi, Centre for Research in Medical Entomology (ICMR)
#4, Sarojini Street, Chinna Chokkikulam, Madurai 625 002, India
e-mail: crmeicmr@icmr.org.in