

## Outdoor resting preference of *Culex tritaeniorhynchus*, the vector of Japanese encephalitis in Warangal and Karim Nagar districts, Andhra Pradesh

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*Background & objectives* : Entomological investigations were carried out in Warangal and Karim Nagar districts of Andhra Pradesh during viral encephalitis outbreak in July 2003.

*Methods* : Adult mosquito collections in outdoor were done using sweep cage method and in indoor with the help of aspirator tube and flash light. Larval collections were done by dipping method. JE virus positivity was tested by IgM capture ELISA test.

*Results* : Outdoor collections revealed presence of *Culex tritaeniorhynchus*, *Cx. bitaeniorhynchus* and *Cx. gelidus* and in indoor collections —*Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *An. vagus* and *An. subpictus*. In the outdoor collections *Cx. tritaeniorhynchus* was predominant (96.3% of total collection). Three samples out of 55 serum samples from human cases and five from contacts showed the presence of antibodies against JE virus.

*Interpretation & conclusion* : *Cx. tritaeniorhynchus*, a known vector of JE is predominant in outdoors and playing a main role in JE transmission in this area. Vector control aimed at the outdoor resting population might limit virus circulation in the mosquito vertebrate host cycle and prevent human infection.

**Key words** Andhra Pradesh – *Cx. tritaeniorhynchus* – outdoor resting habit – sweep cage method – viral encephalitis

The Andhra Pradesh state has been reporting outbreaks of Japanese encephalitis (JE) regularly since 1979<sup>1</sup>. During July 2003 yet another outbreak of viral encephalitis was reported from nine districts of the state— Warangal, Karim Nagar, Nizamabad, Adilabad, Krishna, Nellore, Medak, Nalgonda and Mehboobnagar. A visit was made to Warangal and Karim Nagar districts from where the state health department reported a total of 140 cases and 79 deaths due to viral encephalitis during June 1 to July 13, 2003. As such both the districts are among 10 high incidence

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districts in Andhra Pradesh as far as JE is concerned. This communication reports entomological findings of the investigations carried out in July 2003.

### Material & Methods

Warangal and Karim Nagar districts are in the northern part of Andhra Pradesh state and lie between 17°–19' N and 80°–31' E. Agriculture is the main occupation of the people in the area. The predominant crop is paddy while other minor crops include pulses and maize.

Entomological investigations were conducted in two encephalitis affected villages—Medipathi (PHC Hasanpathi, District Warangal), Uppal (PHC Uppal, District Karim Nagar) and one village (Pagadapalli PHC in Warangal district) which was not affected by this outbreak. Day time outdoor resting adult mosquitoes were collected from low level land vegetation by the recently devised sweep cage method<sup>2</sup>. Land vegetation in the area include widely distributed dense foliage of wild shrubs about 30 cm in height and also low seasonal cash crops like brinjal, pumpkin, etc. Numerous sites of the dense foliages provide suitable resting places for mosquitoes despite the ambient day temperature around 40°C.

### Methodology adopted

*Outdoors* : The collection method involved sweeping a mosquito cage, measuring 12"x12"x12" with its sleeves completely folded (sweep cage), through the vegetation by a series of quick forward, backward, up and down movements for about 1–2 min to disturb the resting mosquitoes and in the process they were trapped in the cage. The sleeve of the cage was immediately folded and the trapped mosquitoes were retrieved from the cage by an aspirator tube. Such attempts were made at least 10 times on vegetation, and the adult mosquito density was then measured as average number of mosquitoes per 10 sweep cages.

*Indoors* : Mosquitoes were also collected from indoors (cattlesheds and human dwellings) following conventional hand catch method with the help of an aspirator tube and a flash-light. The density of mosquitoes was expressed as per man hour (pmh). Presence of immature stages of mosquitoes from the potential breeding habitats in the study areas—paddy nursery beds, irrigated fields, wells, ponds were examined using standard techniques<sup>3</sup>. The data on larval survey were calculated in terms of larval density per dip.

### Results & Discussion

A total of 215 mosquitoes were collected from out-

door situations, resting in extensive dense foliage of low-level wild shrubs and seasonal cash crops in peridomestic area, near paddy nursery bed, etc and 235 mosquitoes from indoor situations in 10 man hour. Outdoor collections revealed three species of mosquitoes—*Cx. tritaeniorhynchus*, *Cx. bitaeniorhynchus* and *Cx. gelidus*, while indoor collections revealed four species—*Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *An. vagus* and *An. subpictus*. In the outdoor collections *Cx. tritaeniorhynchus* was the predominant species (96.3% of the total collection) and in indoor situation *Cx. tritaeniorhynchus* accounted for only 2.9% of the total mosquito collection. *Cx. bitaeniorhynchus* and *Cx. gelidus* were not found resting indoors.

The density of *Cx. tritaeniorhynchus* was found to be high on land vegetation in all the three study villages—Medipathi 50, Pagadapalli 87.5 and Uppal 71.6 per 10 sweep cages each. The indoor density of the species in cattlesheds was 2.3 pmh (village Uppal, Table 1). Both males and females of *Cx. tritaeniorhynchus* were found resting outdoors on the same vegetation. Female specimens collected were in all stages of gonotrophic cycle (Table 2), indicating thereby predominantly outdoor resting habit of the species in the area. Mosquitoes in *Cx. vishnui* subgroup are known to be mainly exophilic<sup>4,5</sup> but during outbreaks of JE they have also been collected from indoor situations<sup>6–10</sup>. Breeding of *Cx. tritaeniorhynchus* was found in abundance (larval density range 7 to 34 per dip) in nursery paddy beds, paddy fields and other large water bodies in the area (Table 3).

The evidence of JE viral activity in the area for the past 10 years (1992–2001) revealed presence of seropositivity to JE virus in humans, pigs and other animals (Source : unpublished report of Veterinary Biological Research Institute, Hyderabad). At the laboratory of National Institute of Communicable Diseases, 55 serum samples from human cases and five from contacts belonging to the present episode were tested by IgM Capture Elisa test for the presence of antibodies against JE virus. Of these, three samples (two cases

**Table 1. Adult mosquito densities in Warangal and Karim Nagar districts, Andhra Pradesh during July 2003**

Mosquito species	Warangal								Karim Nagar			
	Medipathi (PHC Hasanpathi)				Pagadapalli* (PHC Hasanpathi)				Uppal (PHC Uppal)			
	Outdoor**		Indoor		Outdoor**		Indoor		Outdoor**		Indoor	
	Total	PTSC <sup>+</sup>	Total	PMH <sup>++</sup>	Total	PTSC <sup>+</sup>	Total	PMH <sup>++</sup>	Total	PTSC <sup>+</sup>	Total	PMH <sup>++</sup>
<i>Cx. tritaeniorhynchus</i>	38 (100)	50	—	—	76 (95)	87.5	—	—	93 (95.88)	71.66	7 (16.67)	2.33
<i>Cx. bitaeniorhynchus</i>	—	—	—	—	1 (1.25)	2.5	—	—	—	—	—	—
<i>Cx. gelidus</i>	—	—	—	—	3 (3.75)	5	—	—	4 (4.12)	3.33	—	—
<i>Cx. quinquefasciatus</i>	—	—	30 (24.19)	7.5	—	—	18 (26.08)	6	—	—	—	—
<i>An. vagus</i>	—	—	92 (74.19)	20	—	—	51 (73.91)	15	—	—	34 (80.95)	7.66
<i>An. subpictus</i>	—	—	2 (1.61)	0.5	—	—	—	—	—	—	1 (2.38)	0.33
Total	38	50	124	28	80	95	69	21	97	74.99	42	10.32

\*Not affected by the epidemic; \*\*Low level land vegetation; <sup>+</sup>Average no. of female mosquitoes collected per ten sweep cages; <sup>++</sup>Per man hour for female mosquitoes; Figures in parentheses indicate prevalence of a mosquito species in this collection.

**Table 2. Analysis of mosquito species collected from viral encephalitis affected districts, Andhra Pradesh during July 2003**

Mosquito species	Outdoor							Indoor						
	Male	Female				M : F ratio	Male	Female				M : F ratio		
		No.	%*					No.	%*					
			UF	FF	SG				G	UF	FF		SG	G
<i>District Warangal</i>														
<i>Cx. tritaeniorhynchus</i>	59	55	9.1	5.5	50.9	14.5	1.07 : 1	—	—	—	—	—	—	
<i>Cx. bitaeniorhynchus</i>	—	1	—	—	100	—	0 : 1	—	—	—	—	—	—	
<i>Cx. gelidus</i>	1	2	—	50	—	50	1 : 2	—	—	—	—	—	—	
<i>Cx. quinquefasciatus</i>	—	—	—	—	—	—	—	—	48	2.2	20.8	41.6	35.4	0 : 48
<i>An. subpictus</i>	—	—	—	—	—	—	—	—	2	—	—	100	—	0 : 2
<i>An. vagus</i>	—	—	—	—	—	—	—	18	125	28	16	20	36	1 : 6.9
<i>District Karim Nagar</i>														
<i>Cx. tritaeniorhynchus</i>	50	43	11.6	18.6	46.5	23.3	1.16 : 1	—	7	—	—	42.9	57.1	0 : 7
<i>Cx. gelidus</i>	2	2	—	—	100	—	1 : 1	—	—	—	—	—	—	—
<i>An. subpictus</i>	—	—	—	—	—	—	—	—	1	—	—	—	100	0 : 1
<i>An. vagus</i>	—	—	—	—	—	—	—	11	23	17.4	—	52.2	30.4	1 : 2.1

\*Physiological stage/Abdominal condition; UF — Unfed; FF — Full-fed; SG — Semigravid; G — Gravid; M : F — Male : Female.

**Table 3. Mosquito breeding detected in Warangal and Karim Nagar districts, Andhra Pradesh during July 2003**

District/PHC	Village	Type of breeding place	Density/Dip		Mosquito species
			Culicine	Anopheline	
Warangal/Hasanpathi	Pagadapalli	Pond	34	1.8	<i>Cx. tritaeniorhynchus</i> , <i>An. nigerrimus</i>
		Paddy field	7	–	<i>Cx. tritaeniorhynchus</i>
	Medipathi	–	–	<i>Culex</i> * sp, <i>Ae. albopictus</i>	
Karim Nagar/Uppal	Uppal	Paddy nursery	24.6	3.6	<i>Cx. tritaeniorhynchus</i> , <i>An. nigerrimus</i>

\*Species could not be identified.

and one contact) were positive (unpublished report of National Institute of Communicable Diseases). This indicates the recent infection with JE virus in these cases in the affected area.

*Cx. tritaeniorhynchus* is considered to be the important vector of JE in India and virus isolation from this species has been reported from adjoining states of Karnataka<sup>11</sup> and Tamil Nadu<sup>12</sup>. During 2003, entomological investigation was carried out when the viral encephalitis cases and deaths were at their peak in the area. Presence of physiologically active *Cx. tritaeniorhynchus* population in such a high density indicates the possibility of this species being involved in the transmission of current outbreak of viral encephalitis that conforms to the pattern of JE in the area. Thus, in order to control the disease, vector control measures targeted against the physiologically active population of *Cx. tritaeniorhynchus* resting on outdoor vegetation are required. Though the species is known to be mainly exophilic, however during outbreaks when its density rises it has been collected from indoor situations, which might be aiding JE virus transmission in humans. Transmission reduction might, therefore, be achieved in such areas, if the outdoor breeding and resting population of the vector is targeted for its effective control using chemicals or bio-control agents in well-identified outdoor habitats during inter-epidemic periods.

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