Indian J Med Res 129, March 2009, pp 329-332

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Sero-entomological investigations on Japanese encephalitis outbreak in Gorakhpur division, Uttar Pradesh, India

Sir,

Japanese encephalitis (JE) is a non contagious mosquito-borne arboviral disease of major public health importance in Asia. JE is established in India in significant dimensions particularly after sixties when several outbreaks of varying intensity were reported from different parts of India. Outbreaks occur frequently in 14 Asian countries and about 3060 million people live at risk of infection[1,2]. The estimated global burden of JE was 709,000 disability-adjusted life years (DALY) lost in 2003, which is the highest among the encephalitis reported[3].

JE virus infection is widespread and is particularly high in southern States - Andhra Pradesh, Tamil Nadu and parts of Karnataka and Kerala**.** In Uttar Pradesh, the first major JE epidemic occurred in Gorakhpur in 1978 with 1002 cases and 297 deaths reported[4]. Though, many outbreaks were reported in Gorakhpur after the 1978 JE outbreak, in intensity and magnitude, the 2005 epidemic surpassed all previous reported epidemic outbreaks in the country. In 2005, Uttar Pradesh faced a devastating epidemic outbreak of JE mostly confined to Gorakhpur affecting 6061 cases with 1500 deaths followed by another outbreak in 2006 with 2320 cases and 528 deaths. Similarly JE cases in Uttar Pradesh were confined predominantly in Gorakhpur during 2007 reporting 3024 cases and 645 deaths[5]. We conducted this entomological investigation in the Gorakhpur division from August 17 to 30, 2007 and the findings are reported.

Gorakhpur division has seven districts with clayey soil and high water table. The common domestic animals are cattle, pigs, dogs, horses, *etc*. Every village has one or two big tanks and a few ponds[6].

Entomological investigations were conducted in JE affected villages of the following five districts namely, Gorakhpur, Maharajganj, Kushi Nagar, Deoria, and Sidhartha Nagar from August 17, 2007 – August 30, 2007. Adult mosquitoes resting on vegetation and bushes around cattle sheds were collected after dusk with oral aspirator and transported to the laboratory at CRME for identification and enumeration. Wild-caught female mosquitoes were tested for JE virus by using antigen capture ELISA[7].

All the blood specimens obtained from the pediatric ward was collected by hospital staff with informed consent and was tested for JEV-IgM antibodies by using MAC ELISA kit supplied by National Institute of Virology, Pune.

The entomological survey showed the 3010 mosquitoes belonging to 5 genera and 25 species namely, *Culex tritaeniorhynchus, Cx. vishnui, Cx. pseudovishnui, Cx. quinquefasciatus, Cx. gelidus, Cx. whitmorei, Cx. epidesmus, Cx. bitaeniorhynchus, Cx. minutissimus, Cx. fuscocephala, Anopheles subpictus, An. annularis, An. stephensi, An. vagus, An. peditaeniatus, An. barbirostris, Aedes indicus, Ae. scatophagoides, Ae. lineatopennis, Ae. pallidostriatus, Ae. vexans vexans, Ae. jamesii, Mansonia annulifera, Ma. uniformis* and *Armigeres subalbatus* collected from the study area. *Cx. tritaeniorhynchus* was the predominant mosquito present in that area with per man hour (PMH) density of 60.2 and constituted 36 per cent of the total collection followed by *Cx. vishnui* with PMH of 21.8 and formed 13.1 per cent of the total. *Cx. vishnui* subgroup formed 49.6 per cent of the total collection and the PMH density was 82.9. The other species of mosquitoes dominated were the *An. subpictus, Ar. subalbatus*, *Cx. whitmorei* and *Cx. gelidus* (Table). A total of 89 pools of mosquitoes comprising 65 pools of *Culex* species, 6 pools of *Mansonioides* species, 14 pools of *Anopheles* species and 4 pools *Aedes* species were screened by JEV

Table. List of mosquitoes collected during dusk hours in the villages of Gorakhpur Division, Uttar Pradesh

Mosquitoes species	No. collected during dusk hours						PMH
	Gorakhpur North	Gorakhpur South	Kushi Nagar	Maharaj ganj	Deoria	Sidharth Nagar	
Culex tritaeniorhynchus	229	541	132	110	29	42	60.2
Cx. vishnui	36	51	9	253	17	27	21.8
Cx. pseudovishnui	3	2		9	2		0.9
Cx. quinquefasciatus	18	43	29	3	39	2	7.4
Cx. gelidus	31	35	62	27	22	7	10.2
Cx. whitmorei	4		2	181	3		10.6
Cx. epidesmus	2	3	14	16	10		2.5
Cx. bitaeniorhynchus				3	2		0.3
Cx. minutissimus		1					0.06
Cx. fuscocephala	5		3	25	1		1.9
Anopheles subpictus	28	118	22	9	21	182	21.1
An. annularis	2	1		2	1		0.3
An. stephensi		1					0.06
An. vagus	8	4	4	4	1	16	2.1
An. peditaeniatus			18	53			3.9
An. barbirostris	1	1		3			0.3
Aedes indicus	1		2	3	4		0.6
Ae. scatophagoides			1		1		0.1
Ae. lineatopennis	6		14	7	8	2	2.1
Ae. pallidostriatus	2			23	7	77	6.1
Ae. vexans vexans		1		2			0.2
Ae. jamesii			1		4		0.3
Mansonia annulifera				1			0.06
Ma. uniformis	2			14	4	3	1.3
Armigeres subalbatus	37		18		23	151	12.7
Total	415	819	331	749	204	509	

PMH, per man hour

antigen capture ELISA. Of these, two pools were found positive by this method; *Cx. bitaeniorhynchus* - 1 pool with 3 females from Maharajganj and *An. subpictus* - 1 pool with 9 females again from Maharajganj were found to be positive.

Serum samples collected from the suspected JE cases of all age group were tested with MAC ELISA kits obtained from National Institute of Virology, Pune, India. Of the 94 samples tested, 14 were found positive for JEV IgM antibodies. The age group found positive ranged between 1.5 to 13 yr.

Gorakhpur is a hyper-endemic region for JE and the first epidemic of JE was reported in 1978[4]. The seasonality of JE virus (JEV) transmission depends on various factors amongst which the relative abundance of the vector species is one of the important parameters to sustain the transmission[8]. In Gorakhpur, the major JE vectors *Cx. tritaeniorhynchus* was abundant during JE epidemic season and breeding was noticed in rice fields, irrigation channels, fallow fields, rice field pools and grass lands. Until 1961, 16 Culicine species were known from this district[9]. Nine species, including 4 Culicines and 5 Anophelines, were recorded during entomological studies carried out in 1985[10]. A subsequent study conducted in 1988 revealed the presence of 12 species, including 6 Culicines and 6 Anophelines. Sixty seven species of mosquitoes in 11 genera were collected during long-term ecological studies carried out between 1999 and 2000[11].

Paddy is cultivated from July to November, which coincides with the occurrence of JE because encephalitis cases start to increase in August and decline in November, coinciding with peaks and troughs of JE vector population. Earlier studies conducted in Gorakhpur also found rice fields contributing towards the building up of population density of JE vectors[10,12]. The major JE vectors were abundant in all the affected villages during the present investigation. In the previous epidemic investigations also, *Cx.*

tritaeniorhynchus and *Cx. pseudovishnui* were found to be the most abundant species[13-15]. Our study showed the predominance of *Cx. tritaeniorhynchus* followed by *Cx. vishnui.*

In 2007, JEV was found in 2 pools of mosquitoes - *Cx. bitaeniorhynchus* and *An. subpictus*. JE virus was earlier isolated from wild caught *Cx. tritaeniorhynchus* in 1991 outbreak in Gorakhpur[13]. During 2006, JEV was detected from two pools of *Cx. tritaeniorhynchus* and one pool of *Cx. epidesmus* mosquitoes[16]. From the dusk collections *An. subpictus* constituted 12.6 per cent and the PMH density was 21.1 (Table). In Vellore district, Tamil Nadu, this was the most dominant species after *Cx. vishnui* subgroup and was collected throughout the year[17]. JE virus has been isolated from *An. subpictus* in Karnataka[17], Kerala[18] and Tamil Nadu[19]. A two year study in the Cuddalore district of Tamil Nadu showed that the abundance of *An. subpictus*, which was the dominant species among Anopheles in the rice ecosystem, was much lower than that of *Cx. tritaeniorhynchus*. This species appears to play the role of a secondary vector mainly in zoonotic transmission[19].

Cx. bitaeniorhynchus breeding is usually restricted to large ground pools always filled with dense mass of filamentous green algae[20]. This is principally an aviphilic mosquito, which feeds on birds, pigs[19], and humans and also shows a relatively high proportion on cattle feeds[19]. Two JEV isolations have been reported from *Cx. bitaeniorhynchus,* one in Bankura West Bengal[21] and the other in Kolar, Karnataka[17]. In the laboratory this mosquito has been demonstrated to transmit JEV by bite, and has been shown to transmit JEV experimentally between ducks[22], and retained and transmitted the virus by bite for as long as 40 days after infection. This may play a role in the maintenance of JEV in nature that involves birds as reservoirs[19].

Our study showed that the serum samples collected from the suspected patients showed infection in the 15 per cent of the cases, supporting JE infection in Gorakhpur epidemic[22]. Outbreaks in the past were confirmed both clinically and epidemiologically to be of JE[4,15,23,24].

Integrated mosquito control methods need to be applied to tackle this situation in Gorakhpur division. The improved surveillance system developed at the Centre for Research in Medical Entomology (CRME), Madurai could be included as one of the components of early warning system, which can help to predict the impending epidemic well in advance and can predict the probable future course of disease in JE prone areas to identify high risk areas to initiate appropriate control measures[25].

A multi-pronged strategy need to be adopted for prevention of JE outbreaks in endemic areas.

Acknowledgment

Authors thank the health officials Drs. L. P. Rawat, M. K. Gupta, A. K. Rathi, D. K. Srivastava, A. K. Mathur, V. K. Srivastava, J.C. Aggarwal, M.M. Khan, J. K. Rao, R. P. Patel, A.K. Pandey and Sudeshkumar for their co-operation for conducting the investigations at Gorakhpur Division. The technical assistance by Shriyut A. Veerapathiran and S. Venkatesan is acknowledged. Authors acknowledge the assistance by Shri K. Venkatasubramani in preparation of this manuscript.

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