ISSN 0377-4910

ICMR BULLETIN

Vol. 30, No. 4 April, 2000

JAPANESE ENCEPHALITIS VIRUS INFECTION IN MOSQUITOES AND ITS EPIDEMIOLOGICAL IMPLICATIONS

Japanese encephalitis is a serious public health problem in India. The Japanese encephalitis virus (JEV) is maintained in nature by a complex cycle that involves pigs as amplifying hosts, ardeid birds as reservoirs and mosquitoes as vectors. The Culex vishnui subgroup of mosquitoes consisting of Cx. tritaeniorhynchus Giles, Cx. vishnui Theobald and Cx. pseudovishnui Colless have been implicated as major vectors of JE. However in India, JEV has been isolated from 16 species of mosquitoes (Table). Isolation of virus from a species does not provide sufficient proof of its vectorial competence. The main criteria to consider a species of mosquito as a vector are that the population should be high and stable during the epidemic season; long survival rate of the mosquito so that the extrinsic incubation period of the virus is completed; a catholic feeding habit, basically zoophilic in nature having occasional contact with man; repeated isolation of the virus from wild-caught specimens; and finally its competence to support multiplication and successful transmission of the virus in the laboratory. This write-up gives an overview of the studies carried out in India on the species of mosquitoes from which JEV has been isolated, and discusses their possible role in the epidemiology of JE.

Anopheles

Anopheles hyrcanus (gr.) (Pallas) mosquitoes breed primarily in rice fields. Both in the Vellore (erstwhile North Arcot district) and Cuddalore (erstwhile South Arcot district) districts of Tamil Nadu, the prevalence was seasonal but these mosquitoes were present throughout the year in the Bankura district, West Bengal[25]. These mosquitoes are strongly attracted to cattle but were captured in small numbers in pig-baited and chicken-baited traps[3,25]. JEV had been isolated from An. hyrcanus group in Asansol and in Bankura, West Bengal[4,13]. JEV has also been isolated once from An. peditaeniatus (Leicester) in Mandya, Karnataka[2]. An. hyrcanus group could not transmit JEV in the laboratory, although the virus persisted in the mosquitoes for 11 days after the infective feed[1]. Further studies have to be done on Anopheline mosquitoes to explore their role in the epidemiology of JE.

Anopheles barbirostris Van der Wulp is widely distributed in various parts of India. The immatures have been collected from ponds, rice fields, slow streams, disused wells and irrigation channels. In Vellore district adults of this species were abundant during November[3], whereas in Bankura it was prevalent throughout the year[25]. Adults of this species are zoophilic[3]. Only one isolation of JEV

Table. Prevalence of Japanese encephalitis virus infections in mosquitoes

Mosquito species	Prevalence	Blood feeding pattern	Virus isolations	Vector competence	References
An. peditaeniatus	High prevalence in August-September	Zoophilic: mainly on cattle	1 Mandya(Kar)		Dhanda and Kaul 1980[1] Mourya et al 1989[2]
An. barbirostris	Prevalent throughout the year; abundant during November	Pest problem for cattle, attraction ratio buffallo:man 19:1 Zoophilic: cow 89.5%, rarely feeds on man	1 Asansol (WB)		Reuben 1971[3] Chakravarty et al 1975[4] Dhanda and Kaul 1980[1]
An. subpictus	Prevalent throughout the year; higher in hot months than in cool months	Zoophilic: bovines 83%; prefers domestic animals, feeds poorly on pigs and human	5 Kolar(Kar) 1 Kuttanadu(ker) 3 Cuddalore(TN)		Reuben 1971[3] Dhanda and Kaul 1980[1] George et al 1987[5] Dhanda et al 1997[6] CRME Ann. Rep. 1998-99[7]
Cx. tritaeniorhynchus	Prevalent in July-September & October-March; abundant in August,October	Exophilic: strongly attracted to cattle, cattle 88-95%, human 2-4%, pig 0.1-4.4%	9 Vellore(TN) Kolar(Kar) 2 Mandya(Kar) 58 Cuddalore(TN) 7 Kuttanadu(Ker)	Capable to transmit the virus to pigs, mice,ducks&pond herons. Infection rate: 82%, transmission rate 31.6%.	Carey et al 1968[8] Carey et al 1969[9] Reuben 1971[3] Chakravarty et al 1975[4] Soman et al 1986[10] &1977[11] Dhanda et al 1977[12] Banerjee et al 1979[13] & 1984[14] George et al 1987[5] Mourya et al1989[2] Reuben et al 1992[15] Dhanda et al 1997[6] Gajanana et al 1997[16]
Cx. vishnui	More in double crop area; abundant in August-October, November-February	Peak man biting collection: February & September; collected in cattle & pig baits: cattle 85-91%, human 4.5-6%, pig 0-5%	1 Vellore(TN) 1 Asansol(WB) 3 Kolar(Kar) 1 Mandya (Kar) 22 Cuddalore(TN)	Transmitted the virus to chicks	Carey et al 1968[8] Carey et al 1969[9] Reuben 1971[3] Chakravarty et al 1975[4] Dhanda et al 1977[12] Banerjee et al 1984[14] George et al 1987[5] Mourya et al 1989[2] Reuben et al 1992[15] Gajanana et al 1997[16]

Superscript nos. refer to the serial nos. in the reference list

Mosquito species	Prevalence	Blood feeding pattern	Virus isolations	Vector competence	References
Cx. pseudovishnui	High during south west monsoon; peak in August-October,	Cattle 87-89%,human 2.4-6%, pig 0-5.4%	3 Kolar(Kar) 1 Goa	18.7% transmitted	Reuben 1971[3] Chakravarty et al 1975[4] Dhanda et al 1977[12] George et al 1987[5] Naik et al 1990[17] Reuben et al 1992[15] Dhanda et al 1997[6]
Cx. epidesmus	Rare but widespread in northern parts of India during July-November; peak in August	Commonly feed on chicken & pig	1 Bankura(WB)		Banerjee et al 1979[13] Dhanda & Kaul 1980[1] NIV Ann.Rep. 1998-99[18]
Cx. bitaeniorhynchus 1971[19]	Prevalence during and subsequent to rainy season	Attracted to pigs,chiefly aviphilic; birds 57%,cattle 34%,man 5%, pig 3%	1 Bankura(WB) 1 Kolar(Kar)	Capable of transmitting to chicks and ducks; vertically transmitted	Christopher & Reuben Sirivanakaran 1976[20] Dhanda et al 1977[12] Banerjee et al 1978[21] Banerjee et al 1979[13] Dhanda & Kaul 1980[1] Soman & Mourya 1985[22] Reuben et al 1992[15]
Cx. infula	Prevalence during and subsequent to rainy season	Cattle 58%,human 8.3%,birds 8.3%	1 Madurai(TN) 5 Cuddalore(TN)*	Infection rate 60%, transmission rate 47%	Reuben et al 1992[15] Philip et al 1998[23]
Cx. gelidus 1971[19]	Prevalence during and subsequent to rainy season, peak in November-December	Zoophilic & poorly anthropophilic; cattle 95%,human 0.9%,pig 4%	5 Cuddalore(TN) 3 Mandya(Kar)		Christopher & Reuben Reuben 1971[3] Sirivanakaran 1976[20] Mourya et al 1989[2] Reuben et al 1992[15] Gajanana et al 1997[16] NIV Ann.Rep. 1998-99[18]

*JEV antigen detection only

Superscript nos. refer to the serial nos. in the reference list

Mosquito species	Prevalence	Blood feeding pattern	Virus isolations	Vector competence	References
Cx. whitmorei	Widespread throughout India, abundant in rainy season	Cattle 75%, human 25%	1 Vellore(TN) 1 Krishna (AP) 2 Kolar(Kar)		Dhanda & Kaul 1980[1] Banerjee et al 1988[24] Reuben et al 1992[15]
Cx. fuscocephala 1971[19]	Predominant immediately	Highly zoophilic, poorly	1 Mandya(Kar)	Failed to transmit	Christopher & Reuben
	after monsoon	anthropophilic: cattle 77.4%, human 3.2%, pig 3.2%	6 Cuddalore(TN)	JEV	Mahadev et al 1978[25] Mourya et al 1989[2] NIV Ann.Rep 1992[26] Reuben et al 1992[15] Gajanana et al 1997[16]
Cx. quinquefasciatus	Prevalent in many areas, more during March- May	Predilection for man; human 53-63%,cattle 7-14%, pig 1.5%	1 Kolar(Kar)	Capable of transmitting JEV; 63.5% transmission	Banerjee et al 1977[27] Mourya et al 1989[2] Reuben et al 1992[15]
Ma. annulifera	Prevalent through out the year in low profile, more in September, October	Endopilic: man landing rate 24.67(1.75-65); 2 peaks – 2400-100h & 400-500h; anthropophilic index 0.98, feeding:human 58.2%, pig 0.7%	1 Dibrugarh(AS) 1 Kuttanadu(Ker)		Chakravarty et al 1981[28] Narasimham et al 1988[29] Pradeep et al 1989[30] CRME Ann.Rep. 1998-99[7]
Ma. indiana	Through out the year in low profile	24% human bait,3% bovine bait, 2 biting peaks: 2000-2100h & 300-400h, man landing rate 0.47, Feeding: human 23%,pigs 11%	1 Kuttanadu (Ker) 2 Kuttanadu(Ker)		Pradeep et al 1989[30] Dhanda et al 1997[6] CRME Ann.Rep. 1998-99[7]
Ma. uniformis	Prevalence high in November, predilection for outdoor resting	Exophagic: dusk biting 1800-1900h; 45% in first 3 hours, feeding: human 12%, pig 10%	3 Kuttanadu(Ker) 1 Mandya(Kar)*		Mourya et al 1989[2] Pradeep et al 1989[30] Dhanda et al 1997[6]

*JEV antigen detection only
Superscript nos. refer to the serial nos. in the reference list.

has been made from An. barbirostris in Asansol, West Bengal[4]. The vectorial potential of this species has not been studied.

Anopheles subpictus Grassi breeds profusely in water collections and fallow rice fields[1]. In southern India the larval incidence was high throughout the year. In Vellore district, this was the most dominant species after Cx.vishnui group and was collected throughout the year[3]. JEV has been isolated from An. subpictus in Karnataka[5], Kerala[6] and Tamil Nadu[7]. 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. The adult density was higher in the hot months than in the cool months. An. subpictus are strongly zoophilic feeding mostly on bovines (83%) and poorly on pigs and human. JEV infection in this species of mosquito was 3.47/1000[7]. An. subpictus appears to play the role of a secondary vector mainly in zoonotic transmission.

Culex

Of the subgenus Culex, members of the Cx. vishnui subgroup viz. Cx. tritaeniorhynchus, Cx. vishnui, Cx. pseudovishnui are extremely common, widespread and mainly breed in paddy fields and the abundance is related to rice cultivation. Blood meal analysis showed that these mosquitoes were principally cattle feeders though human and pig feeding was also recorded in villages near Madurai and in Nallur PHC of Cuddalore district[15] and in Dibrugarh, Assam[31]. The maximum isolation of JEV was from the Cx. vishnui subgroup [2,4-6,8,13,16,17,32] of mosquitoes which were shown to be capable of transmitting the virus in the laboratory[9,11,12,33]. Cx. vishnui subgroup has been recognized for many years as the major vectors and play an important role in the epidemiology of JE in India.

JEV has also been isolated from members of the Cx. bitaeniorhynchus subgroup of which Cx.bitaeniorhynchus Giles and Cx. infula Theobald are common throughout India. Cx.epidesmus Theobald is widespread in the northern parts of India. The adults were collected in all types of dusk collections in and around cattle sheds and pigsties[18]. This species was captured in bird-baited traps as well as feeding on pigs and man[1]. Only one isolation of JEV was recorded from Cx. epidesmus in Bankura, West Bengal[13]. The vector potential of this species needs to be studied.

Culex bitaeniorhynchus breeding is 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[15], humans and also shows a relatively high proportion of cattle feeds[19]. Two JEV isolations have been reported from Cx. bitaeniorhynchus, one in Bankura West Bengal[13] and the other in Kolar, Karnataka[21]. In the laboratory it has been demonstrated to transmit JEV by bite[21,22], and has been shown to transmit JEV experimentally between ducks and chicks[12,21]. This mosquito retained and transmitted the virus by bite for as long as 40 days after infection[1]. This may play a role in the maintenance of JEV in nature that involves birds as reservoirs.

During the rainy season immature stages of Cx. infula were collected from paddy fields in Madurai and Cuddalore districts of Tamil Nadu. Of the total mosquitoes collected in Madurai district, Cx. infula comprised 13.4% in dusk collection and 34.5% in man-biting collections. Cx. infula mainly feeds on cattle, human and birds[15]. One isolation of JEV from Cx. infula in Madurai and five JEV antigen detections from Cuddalore district have been reported. Vector competence study showed infection and transmission rates as 60 and 47% respectively[23]. This species may play a role in JE transmission because of its seasonal abundance and man biting habits during JE season besides its competence to transmit JEV.

Culex gelidus Theobald species prefers marshy depressions containing abundant aquatic vegetations[20] and was abundantly available during the north-east monsoon season in Vellore district[3]. Adults were found closely associated with man and domestic animals and most of them feed on bovids[19] and pigs[15]. JEV has been isolated three times in Mandya district of Karnataka[2] and 5 times in Cuddalore district of Tamil Nadu[16]. Though this mosquito is only seasonal due to its feeding habit and infection rate, they may have an important role in the zoonotic cycle. Its vectorial potential is to be studied.

Culex whitmorei (Giles) is widely spread throughout India. It breeds in freshwater ground pools, including puddles and ditches in rice fields, containing numerous grasses. Prevalence of adults shows a peak usually towards the end of the rainy season. Cx. whitmorei is predominantly a cattle feeder[15] but it also feeds on pigs, birds and men[1]. Four isolations of JEV have been made in Cx. whitmorei from widely separated localities, two isolations from Kolar district[24] and one each from Vellore district, and Krishna district, Andhra Pradesh[1]. Vector competence of this mosquito is to be studied to know its role in the transmission of JEV.

JEV has been isolated from wild-caught Cx. fuscocephala Theobald. This species mainly breeds in ground pools and the adults were collected inside cattle sheds, pig baited traps and in light traps in Bankura[25]. In Vellore district, adults were collected mainly on cattle or buffaloes. Blood meal analysis showed that they mainly feed on bovids, and rarely on pigs[19]. JEV has been isolated from Cx. fuscocephala once in Mandya district[2] and six times in Cuddalore district[16]. But the experimental transmission studies on Cx. fuscocephala failed to demonstrate JEV transmission though the virus was picked up[26]. Like Cx. gelidus this mosquito may have an important role in zoonotic cycle.

Culex quinquefasciatus Say is the most common domestic species in urban, semiurban and rural areas. It is strongly anthropophilic (53.2-62.7%); 7-14.7% cattle feeding and 1.5% feeding on pigs were also observed[15]. A single isolation of JEV was made from Cx. quinquefasciatus in Kolar district in 1986[2]. Cx. quinquefasciatus (Culex fatigans)has been shown to be capable of transmitting the JEV in the laboratory[27]. But the epidemiology of JE does not fit into the known information regarding the ecology and behaviour of Cx. quinquefasciatus.

Mansonia

Mansonia species, the vectors of brugian filariasis breed in water with floating vegetation like Pistia,Salvinia and Eichhornia[30]. JEV was isolated from Ma. annulifera in Dibrugarh, Assam[28]. In a recent outbreak of JE in Kerala, three isolations were made from Ma. uniformis and one from Ma. indiana[6]. JE antigen was detected in Ma. uniformis from Mandya district, but isolation of the virus was not achieved[2]. Isolations of the JEV from these species of mosquitoes indicate that these species have access to viraemic hosts. Studies are required on the vectorial potential of Mansonia species for JEV transmission.

Though JE is a vaccine preventable disease, hundreds of children suffer from JE. Epidemics of JE continue to occur in different parts of the country and the disease is spreading to new areas. Further studies on the vector competence of some of these species are necessary before any conclusion can be drawn about their role in the epidemiology of JE in India. There is lack of epidemiological data from different endemic areas. Effective surveillance systems are urgently required to identify the vector mosquitoes in the eastern and north-eastern parts of India and to monitor JE activity in vectors from different parts of India.

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