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Japanese encephalitis virus isolation from mosquitoes during an outbreak in 2011 in Alappuzha district, Kerala

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Japanese encephalitis (JE) virus (JEV) is a major mosquito-borne encephalitic flavivirus of rural eastern, southeastern and southern Asia. Outbreaks of JE have occurred in many states in India[1]. An explosive insular outbreak of meningoencephalitis, mainly in children, occurred during early 1996 in the Kuttanad area of Alappuzha district, Kerala[2]. During the outbreak in May 2011, 24 cases were recorded between January and May 2011; one in January, one in February, four in March, four in April, and 14 in May. The outbreak due to JE was diagnosed by the Centre for Research in Medical Entomology, Madurai, using JE MAC-ELISA kit obtained from National Institute of Virology, Pune (Unpublished data). We report here recovery of JEV from field collected mosquitoes during the outbreak.

Out of 580 wild-caught mosquitoes from 124 pools tested for virus using antigen capture ELISA and an insect-bioassay (inoculation into *Toxorhynchites splendens* larvae and identification by IFA using Japanese encephalitis (JE) virus-specific monoclonal antibody), four flavivirus isolations were made, of which 2 (50%) were identified as JE virus, one each from *Culex tritaeniorhynchus* and *Cx. gelidus*.

Kerala is an Indian state with a total area of 38,863 km^2 and population of 31,838,619. Alappuzha lies at the western part of Kerala. It is the smallest district of the Kerala state. The latitude and longitude of Alappuzha district are 9°30′ N, 76°23′ E, respectively. Alappuzha district in Kerala was worst-affected with fever cases during May 2011. Mosquitoes were sampled on May 10–12, 2011 in Kuthirapanthi, Gynagiri, Ashramam, and Champakulam villages, the worst-affected areas of Alappuzha district.

Adult mosquitoes were collected by hand catch method using aspirator and torch light. During dusk hours, mosquitoes were collected for one hour in and around cattlesheds (dusk collection). Indoor resting collections were done inside the human dwellings during 0800–1000 hrs (indoor collection). Outdoor resting mosquitoes were collected under the vegetation around the cattlesheds using drop net method (outdoor collection). After collection these were transported to the field laboratory, lightly anaesthetized with ether, identified, and sorted on ice into pools of 1–50 specimens/pool. Unfed mosquitoes were pooled on the same day of collection, whereas engorged female mosquitoes were held for 48 h for digestion of blood meals before pooling.

Mosquito pools were stored at –80°C until processed for virus detection and isolation as described by Gajanana *et al*[3–4]. Two systems were used :

(i) *Antigen capture ELISA:* Monoclonal antibody 6B4A-10 (reactive against all viruses in JE/WN/SLE/MVE complex) was used as capture antibody and monoclonal antibody peroxidase conjugate SLE MAB 6B6C-1 (reactive against all flaviviruses) as detector antibodies (supplied by Dr T.F. Tsai, Centers for Disease Control and Prevention, Fort Collins Co., USA).

(ii) *Insect bioassay: Toxorhynchites splendens* mosquito larvae were inoculated intracerebrally, incubated for 7–10 days at 29°C and then tested by the indirect immunofluorescence assay (IFA) on head squeeze preparations (Toxo-IFA). Smears were tested with JEV-specific monoclonal antibody, MAB 112 (supplied by Dr Kimura Kuroda, Tokyo Metropolitan Institute of Neurosciences, Japan), and detected by FITC conjugated antimouse immunoglobulin (Dakoppats, Denmark).

Virus infection rate in mosquitoes was expressed as minimum infection rate (MIR) per 1000 females tested. In total, 580 female mosquitoes representing seven species were collected. *Culex tritaeniorhynchus* Giles (73.28%), *Cx. gelidus* Theobald (17.07%), and *Armigeres subalbatus* Coquillett (7.07%) comprised 97.42% of the total catch. The rest of the species, namely *Mansonia annulifera* Theobald, *Ma. uniformis* Theobald, *Cx. bitaeniorhynchus* Giles, and *Cx. quinquefasciatus* (Say) comprised 2.58% (Fig. 1).

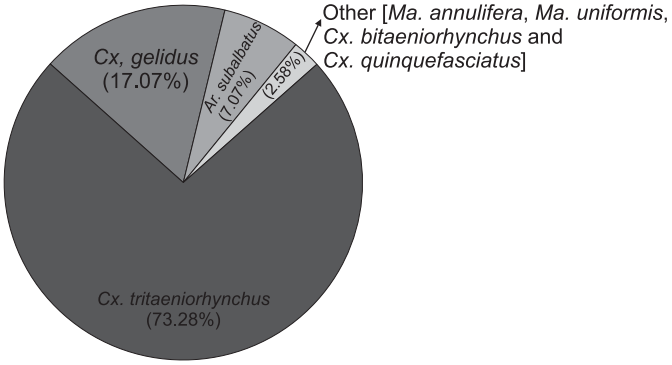

Fig. 1: Species composition of mosquitoes collected in Alappuzha district, Kerala during January to May 2011.

A total of 580 adult mosquitoes were tested for virus in 124 pools by ELISA and four pools were found positive for flavivirus antigens (Table 1). The four ELISA positive pools were tested by the Toxo-IFA system using JEV-specific MAB 112 and of these, two isolates were confirmed as JEV (Fig. 2). Of the two JEV isolations one was from *Cx. tritaeniorhynchus*, and another was from *Cx. gelidus*, both collected in dusk collection from Kuthirapanthi village, one of the affected area.

Table 1. JE virus detection from mosquitoes in Alappuzha district, Kerala during January to May 2011

S.No.	Mosquito species	No. of pools tested (Total no. of mosquitoes)	Number positive	MIR/ 1000
1.	*Cx. tritaeniorhynchus*	86 (425)	2	4.71
2.	*Cx. gelidus*	23 (99)	2	20.20
3.	*Ma. annulifera*	2 (8)	–	–
4.	*Ma. uniformis*	2 (5)	–	–
5.	*Ar. subalbatus*	9 (41)	–	–
6.	*Cx. bitaeniorhynchus*	1 (1)	–	–
7.	*Cx. quinquefasciatus*	1 (1)	–	–
	Total	124 (580)	4	6.90

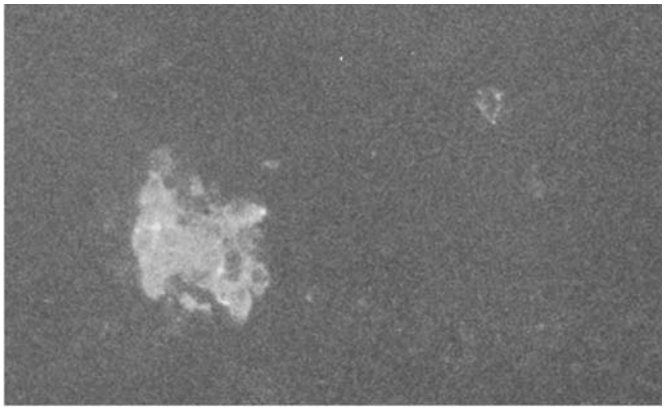

Fig. 2: Results of *Toxorhynchites splendens* immunofluorescent assay (Toxo-IFA).

DISCUSSION

As in the present investigation, Philip Samuel *et al*[5] also reported in Gorakhpur, Uttar Pradesh, India during JE outbreak investigation that the major JE vector *Cx. tritaeniorhynchus* was the most abundant species in the affected villages. *Culex tritaeniorhynchus* is universally recognized as the primary vector of JE and the largest number of isolations have been made from this species[2, 4, 6]. A good number of JEV isolations have also been made from *Cx. gelidus* in India[4, 7–8]. During investigation of an outbreak of JE in the Torres Strait, Australia, one isolate of JEV was obtained from *Cx. gelidus*, which was the first isolate of JEV from *Cx. gelidus* in the Australian region[9]. In this study also, one JEV isolation from *Cx. gelidus* is reported. It is considered to be one of the most important vectors of JE elsewhere, and isolations of JEV strains have been reported from wild caught *Cx. gelidus* in Malaysia[10]. Gingrich *et al*[11], studied the vector ecology in urban JE in 1986–87 in suburban communities of Bangkok and found *Cx. tritaeniorhynchus* and *Cx. gelidus*, the major vector mosquitoes and the MIR of *Cx. gelidus* (0.47) was higher than *Cx. tritaeniorhynchus* (0.17). In Cuddalore district, number of JEV isolates was higher in *Cx. tritaeniorhynchus* than *Cx. gelidus* but MIR of *Cx. gelidus* (0.52) was higher than *Cx. tritaeniorhynchus* (0.28). In this study also, the MIR/1000 of *Cx. gelidus* was higher (20.20) than *Cx. tritaeniorhynchus* (4.71). JEV was isolated from *Cx. gelidus* during a JE epidemic in the dry zone of Sri Lanka[12]. Natural vertical transmission of JEV in *Cx. gelidus* was also noticed in Cuddalore district[13]. In this investigation, the above two species were involved in the transmission of JEV in Alappuzha district, Kerala.

Outbreaks in the past in Kerala were also confirmed both clinically and epidemiologically to be of JE[2]. Integrated mosquito control methods need to be applied to tackle this situation in Kerala. The improved surveillance system developed at the Centre for Research in Medical Entomology, 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[14].

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