Journal of the American Mosquito Control Association, 10(2):228–229, 1994

ISOLATION OF JAPANESE ENCEPHALITIS VIRUS FROM *CULEX SITIENS* MOSQUITOES IN SELANGOR, MALAYSIA

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ABSTRACT. Isolation of Japanese encephalitis virus (JEV) from mosquitoes in Sabak Bernam, Selangor, Malaysia, was attempted. An aliquot of homogenate from each pool of mosquitoes, 50 per tube, was inoculated into *Aedes albopictus* clone C6/36 cells for virus isolation. Each cell culture was tested for the presence of viral antigen by immunoperoxidase staining using an anti-JEV polyclonal antibody. Out of 4 *Culex sitiens* mosquito pools, 2 pools were positive for JEV by cell culture. Presence of JEV genome in the cell cultures for *Cx. sitiens* was confirmed by using reverse transcriptase–polymerase chain reaction and JEV-specific primers. This is the first report on the isolation of JEV from *Cx. sitiens*.

After one positive case of Japanese encephalitis virus (JEV) was confirmed from Kampong Pasir Panjang, Sabak Bernam District, Selangor, Malaysia, in May 1992, mosquitoes were collected for virus isolation tests from that area. Adult mosquitoes were collected using CDC battery-operated light traps with CO_2. The traps were operated each night from 1800 to 0700 h. The mosquitoes caught in trap bags were killed on dry ice and then sorted according to species, recorded, and pooled. Pools of mosquitoes, 50 per tube, were then placed in liquid nitrogen and returned to the laboratory for virus isolation attempts. All bloodfed mosquitoes were discarded.

Each pool of mosquitoes was homogenized with 2 ml of 0.2% bovine plasma albumin in phosphate-buffered saline, pH 7.2, and was centrifuged at 3,000 rpm for 15 min at 4°C. An aliquot of each supernatant was inoculated into *Aedes albopictus* clone C6/36 cells (Igarashi 1978). After 7 days incubation at room temperature, C6/36 cell smears were prepared from each culture on a 12-well microscope slide to examine for the presence of viral antigen by immunoperoxidase staining using an anti-JEV polyclonal antibody.

A total of 256 pools belonging to 35 species and 8 genera were collected for virus isolation. Of these, two pools (601 and 739) of *Culex sitiens* Wiedemann were positive for JEV by tissue culture. Reverse transcriptase–polymerase chain reaction (RT-PCR) was applied to confirm presence of JEV genome in the cell cultures. Ribonucleic acid (RNA) was extracted from the cell cultures by a procedure previously described by Chomczynski and Sacchi (1987). The amplification reaction was performed by combining the reverse transcription of viral RNA and the subsequent DNA polymerase amplification in a single tube. Target RNA was amplified in 100-μl volumes containing the following components: 50 mM KCl, 10 mM Tris (pH 8.8), 1.5 mM $MgCl_2$, 0.1% Triton X 100, 200 μM each of the 4 deoxynucleotide triphosphates, 5 mM dithiothreitol, 50 pmol each of primers 1 and 2, 8 U of RT (Promega), and 2.5 U of DynaZyme (Finnzymes OY). Two primer pairs, (JBE-1 and JBE-2) ATGACTAAAAAACCAGGAGG and CTTGCGAGCCACATGATTGA or (JEN3S and JEN3R) AACTCAACCGCAAGTCCTAT and GACCCTGCTCGCACGGAAGT were applied in the RT-PCR (Eldadah et al. 1991, Tanaka et al. 1993). The reactions were allowed to proceed in a Perkin-Elmer-Cetus thermal cycler programmed to incubate for 1 h at 42°C and then to proceed with 35 cycles of denaturation (95°C, 2 min), primer annealing (53°C, 1 min) and primer extension (72°C, 3 min). The correct size of the DNA product was obtained for JaGArO1 and 601 and 739 after amplification with each of primer pairs A or B (Fig. 1).

Japanese encephalitis virus is common over a wide part of Asia. The natural transmission cycle involves *Culex tritaeniorhynchus* Giles and other closely related *Culex* mosquitoes that breed primarily in rice fields and feed on humans in the early evenings (Shope 1990). This is the first isolation of JEV from *Cx. sitiens*. This species is a brackish pool breeder and is found near the sea coast. It is found as far as East Africa, throughout the whole of the Oriental region to Australia and Fiji.

In this area the number of *Cx. sitiens* per trap night varied from 0 to 67.3 and the number per human per night varied from 0.3 to 4.2. It was found in large numbers during the month of September, which is the beginning of the rainy season. *Culex sitiens* comprised 3.2% of the total collections in our study area.

Culex sitiens is a vicious biter and bites both indoors and outdoors. Colless (1959) has re-

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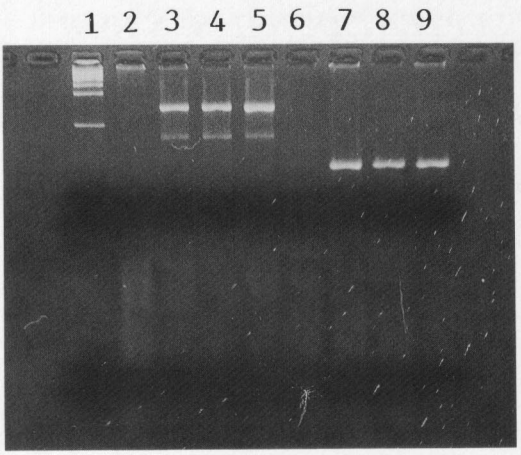

Fig. 1. Electrophoresis in 3% agarose gel (RT-PCR). Size of PCR product (JBE-1 and JBE-2), 350 bp. Size of PCR product (JEN3S and JEN3R), 116 bp. Lane 1: DNA marker; Lane 2: Control (JBE-1 and JBE-2); Lane 3: 601 (JBE-1 and JBE-2); Lane 4: 739 (JBE-1 and JBE-2); Lane 5: JaGArO1 (JBE-1 and JBE-2); Lane 6: Control (JEN3S and JEN3R); Lane 7: 601 (JEN3S and JEN3R); Lane 8: 739 (JEN3S and JEN3R); Lane 9: JaGArO1 (JEN3S and JEN3R).

ported that *Cx. sitiens* feeds primarily on birds and pigs. We have also found the same to be true. In our present study, 140 blood smears of *Cx. sitiens* were tested for host preference by a modification of the countercurrent immunoelectrophoresis (CCIE) method of gel precipitation by Culliford (1964). Of these 81.4% (114) fed on pigs, 9.3% (13) on cows and humans, 4.3% on pigs and humans, and 1.4% (2) on humans. Because this is the first report of the isolation of JEV from *Cx. sitiens,* further work is being carried out to determine its capability in transmitting JEV.

We thank Dato' Dr. M. Jegathesan, Director, Institute for Medical Research for his permission to publish this paper, Professor H. Tanaka, chief adviser, Japanese International Cooperation Agency (JICA) for his constructive comments, Professor A. Igarashi and Dr. K. Morita from Nagasaki University, Japan, for their guidance and supply of primers, and Ms Zaridah for collection and processing of mosquitoes. This project is funded by Malaysian Government R&D Grant No. IMR 92-6 and JICA.

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