JAPANESE ENCEPHALITIS VIRUS ISOLATED FROM SEVEN SPECIES OF MOSQUITOES COLLECTED AT SEMARANG REGENCY, CENTRAL JAVA

Ratna Tan*, Sustriayu Nalim**, H. Suwasono** and G.B. Jennings*

ABSTRAK

Suatu penelitian telah dilakukan untuk mengisolasi arbovirus dari nyamuk yang ditangkap dari rumah penduduk dan kandang temak desa Susukan, kecamatan Ungaran, kabupaten Semarang. Pada periode penelitian yang dilakukan selama 2 tahun, isolasi virus hanya didapatkan pada bulan Januari dan Februari. Virus Japanese encephalitis (JEV) adalah satu-satunya virus yang dapat diisolasi. Mayoritas isolat JEV (72%) berasal dari nyamuk yang ditangkap di kandang temak. Isolat JEV ini berasal dari 7 spesies nyamuk, dengan frekuensi Culex quinquefasciatus yang paling tinggi (11.5%). Empat vektor JEV baru yang potensial di Indonesia adalah Cx.bitaieniorhynchus, Cx.quinquefasciatus, Anopheles kochi dan Armigeres subalbatus. Ini adalah laporan pertama tentang isolasi JEV dari An.kochi dan Ar.subalbatus di Asia.

INTRODUCTION

Arthropod-borne viruses (arboviruses) are responsible for causing undiagnosed symptomatic and asymptomatic infections in human and animals1. In Indonesia, human and animal serological surveys have detected antibodies against several arboviruses. Olson et al.2 used hemagglutination-inhibition (HI) assays to demonstrate antibodies to Japanese encephalitis virus (JEV), Zika virus, Chikungunya virus (Chik) and Ross River virus (RR), and Tesh et al3 demonstrated neutralization antibody to Chik, RR, Getah, Bebaru and Sindbis viruses. The existence of dengue virus4, Chik, (unpublished data) and JEV5 has been confirmed by viral isolation.

Previously, Suwasono et. al.6 conducted a risk-assessment study in Ungaran in response to historical reports about many patients with fever of unknown origin at the local health clinic. They reported the collection of 5 species of Culex, 2 species of Aedes and 1 species of

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Armigeres in cattle shelters and nearby houses in the area, suggesting that these species might be possible arbovirus vectors.

This concurrent portion of the study was designed to determine if the collected mosquitoes were possible arbovirus vectors. This is a report of the identification of 4 new potential JEV vectors in Indonesia and confirm the existence of JEV circulation in Central Java.

MATERIALS AND METHODS

Mosquito collections.

Mosquitoes were collected once weekly by mouth aspirator at the villages of Susukan, Ungaran district, Semarang regency, between September 1986 and August 1988. Briefly, resting collections were made during daylight hours inside houses and during the evening in cattle shelters. Female mosquitoes were held for two days to permit digestion of blood meals, then chilled, identified, and individually separated into pools by species, collection site and collection date with a maximum of 50 mosquitoes per pool.

Preparation of mosquito pools

Pools were ground in a chilled ten broeck grinder in 3 ml. of chilled phosphate and tricine (0.01 M. each), buffered saline (PBS) pH 7.8 containing 0.8 % bovine albumin, 100 units of Penicillin and 100 mg of Streptomycin. The resulting mosquito suspensions were centrifuged at 3000 rpm for 20 minutes at 4°C. The supernatant was filtered, with 0.2 micron filter, and stored at -60°C for virus isolation.

Virus isolation

Mosquito-pool suspensions were inoculated onto Toxorhynchites splendens mosquito cells (TRA-284) obtained from CDC, San Juan, Puerto Rico. Viral isolates were identified as described previously, using alphavirus and flavivirus specific monoclonal antibodies, and then confirmed with viral specific monoclonal antibodies supplied by CDC, Fort Collins, USA.

RESULTS

A total of 1385 mosquito pools from resting collections in human dwellings and cattle shelters were assayed with 25 (1.8%) positive for virus. Table 1 shows the number of mosquito species assayed and the results of virus isolation attempts. JEV was the only arbovirus isolated from the mosquitoes. The majority (72%) of the JEV isolates were from mosquitoes collected in the cattle shelters. Only one species collected from the house, Cx. quinquefasciatus, was positive (7 pools) for virus. In the cattle shelters, JEV isolations were made from several species, but Cx. quinquefasciatus had the highest frequency (11.5%). Overall amongst all 4 genera studied, Culex species had the highest frequency (1.23%). Although mosquitoes were collected during the 2-year period, virus isolations were made only from pools collected during the wet months of January and February 1987.
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JEV VIRUS ISOLATION

<table>
<thead>
<tr>
<th>MOSQUITO SPECIES</th>
<th>IN HOUSES</th>
<th>IN CATTLE SHELTERS</th>
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<tbody>
<tr>
<td></td>
<td>No of pools tested</td>
<td>No of positive pools</td>
</tr>
<tr>
<td>Cx. fuscocephalus</td>
<td>10</td>
<td>–</td>
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<td>Cx. tritaeniorhynchus</td>
<td>4</td>
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<td>Cx. vishnui</td>
<td>60</td>
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<td>Cx. bitaeniorhynchus</td>
<td>31</td>
<td>–</td>
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<tr>
<td>Cx. p. quinquefasciatus</td>
<td>307</td>
<td>7</td>
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<tr>
<td>An. vagus</td>
<td>16</td>
<td>–</td>
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<tr>
<td>An. indefinitus</td>
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<td>An. maculatus</td>
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<td>An. annularis</td>
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<td>An. kochi</td>
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<td>An. barbirostris</td>
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<td>An. aconitus</td>
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<td>Ae. aegypti</td>
<td>87</td>
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<td>Ae. albopictus</td>
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<td>Ae. polcillus</td>
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<tr>
<td>Ae. caecus</td>
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<td>–</td>
</tr>
<tr>
<td>Armigeres subalbatus</td>
<td>35</td>
<td>–</td>
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</tbody>
</table>

DISCUSSIONS

Both the role of mosquitoes as the vector and of vertebrates as the amplifying host, are critically important for the natural transmission of arboviruses. Mosquitoes of the genus Culex\(^5\) and Anopheles\(^8\) had previous JEV isolates in Indonesia. In this study, 4 new potential JEV vectors in Indonesia were identified: Cx. bitaeniorhynchus, Cx. quinquefasciatus, An. kochi and Ar. subalbatus. Additionally, An. kochi and Ar. subalbatus are reported as potential JEV vectors for the first time in Asia\(^9\).

Percentages of all mosquitoes collected were higher in cattle shelters than in houses throughout this study and were not affected by the rainy season\(^6\). Isolated virus was from mosquitoes collected during the normally wet months of January and February. Six (24%) of the JEV isolates came from Cx. tritaeniorhynchus and Cx. fuscocephalus, considered to be among the principal vectors of JEV throughout the rice-growing areas of Asia\(^9\). This may identify the active transmission period, and the time period of greatest risk to humans. Cx. quinquefasciatus, the only JEV infected mosquito found in both houses and cattle shelters, might pose the greatest threat to man. Although not all mosquito species in this study were found to contain virus, their

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presence may indicate the potential for increased risk of virus transmission in the area. Several of the species examined are known vectors to humans of other arboviruses, for example *Ae. aegypti* and *Ae. albopictus* for dengue and Zika\textsuperscript{10,12} and *Ae. aegypti* for Chik\textsuperscript{11}.

Although JEV was isolated, there have been no reports of JEV clinical infection in Indonesia. Suharyono\textsuperscript{13} has proposed several reasons for the absence of JEV clinical cases in Indonesia. One possible reason is the lack of pigs in Indonesia as the amplifying host, because the country is predominantly Muslim. In this study, we found that cattle are potential amplifying hosts since numerous JEV infected mosquitoes were found in the cattle shelter. Chen et al.\textsuperscript{14} recently identified a new JEV genotype in Indonesia. Whether this genotype has decreased virulence is unknown, but if true, could account for the lack of clinical cases.

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