First time Detection of Japanese encephalitis Virus Antigen in dry and Unpreserved Mosquito *Culex tritaeniorhynchus* Giles, 1901, from Karnal district of Haryana state of India

B.P. Das,* S.N. Sharma,* L. Kabilan,** S. Lal* and V.K. Saxena*

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**ABSTRACT**

Japanese encephalitis virus (JEV) antigen has been detected by antigen capture enzyme linked immunosorbent assay (ELISA) in dry specimens of the mosquito *Culex tritaeniorhynchus* Giles, 1901, collected from Karnal district of Haryana state in northern India. These mosquitoes were stored in dry condition for 20 months, at room temperature, before processing. The procedure of detecting JEV infection in long time stored, dry vector mosquitoes, has important application in the surveillance of Japanese encephalitis.

**Keywords:** *Culex tritaeniorhynchus*, JEV-antigen detection, northern India, antigen-capture enzyme immunoassay, dry mosquito, room temperature, long period of storage.

**INTRODUCTION**

Detection of virus in mosquitoes is an important component of outbreak investigations to confirm the diagnosis of the disease in the outbreak. In developing countries due to inadequate facilities of storage of mosquitoes at sub zero temperatures, detection of virus in mosquitoes is generally limited to a few laboratories where facilities exist. Unpreserved dry mosquitoes stored at room temperature for 30 days were used to detect Japanese encephalitis (JE) virus-antigen in *Culex tritaeniorhynchus*. This communication reports the detection of JEV virus in *Culex tritaeniorhynchus* from district Karnal, Haryana state in northern India, which is endemic for JE, and where the disease struck for the first time in 1990.3

**MATERIALS AND METHODS**

Adult mosquitoes were collected from rural and periurban areas of district Karnal (2001-2003), identified and stored in dry state at room temperature (27°C ± 2°C) for a period ranging from one month to 20 months at Centre for Medical Entomology and Vector Management, National Institute of Communicable Diseases (NICD). Mosquito...
pools comprising of unfed *Culex tritaeniorhynchus* and *Cx. perplexus* were transported in dry condition, without cold chain facility to the Centre for Research in Medical Entomology (CRME), Madurai, India, where viral antigen detection facilities were adequate. The material was processed using an antigen-capture enzyme linked immunosorbent assay (ELISA).3,4 For this assay, monoclonal antibody (MAb) 6B4A-10 reactive against JEV was used as capture antibody and MAb-peroxidase conjugate SLE MAb 6B6c-1 (reactive against all flaviviruses) as detector antibody. The ELISA plate contained known positive (JEV infected suckling mouse brain homogenate) and negative (homogenate of uninfected laboratory reared adult mosquito pools) controls.

**RESULTS AND DISCUSSION**

A total of 17 pools (787 unfed female mosquitoes) representing two species were processed for detection of JEV infection by ELISA. Of these, 2 pools (135 mosquitoes) belonged to *Culex perplexus* and 15 pools (652 mosquitoes) belonged to *Cx. tritaeniorhynchus* (Table 1). Mosquito pool was considered positive for virus antigen if its optical density (OD) was greater than or equal to mean +4 standard deviation (SD) of the OD of the normal laboratory reared mosquito pools.

Out of 4 pools of *Cx. tritaeniorhynchus* tested (652 mosquitoes), one pool was found positive for JE virus antigen (OD = 0.078). The cut off OD value was 0.073 (mean and SD of negative controls were 0.065855 and 0.00172240 respectively). OD value of positive control was 0.157. The minimum infection rate (MIR) of *Cx. tritaeniorhynchus* was 6.06. The mosquitoes of the positive pool were collected from indoor situation during evening hours in periurban locality of Karnal town in October, 2002 and stored (27°C ± 2°C) for a period ranging 20 months.

Table 1. Mosquitoes collected from Karnal district (Haryana) and tested for JE virus Antigen by ELISA

<table>
<thead>
<tr>
<th>Mosquito species</th>
<th>Locality</th>
<th>No. of pools tested/No. of adults/ No. of pools positive</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Culex tritaeniorhynchus</em></td>
<td>Karnal periurban area</td>
<td>4/165/1</td>
</tr>
<tr>
<td></td>
<td>Kutail Kalan, PHC Kutail</td>
<td>3/138/0</td>
</tr>
<tr>
<td></td>
<td>Kambopura, Indri Block</td>
<td>2/100/0</td>
</tr>
<tr>
<td></td>
<td>Karnal periurban area</td>
<td>2/81/0</td>
</tr>
<tr>
<td></td>
<td>Kharkhali, PHC Madhuvan,</td>
<td>4/168/0</td>
</tr>
<tr>
<td><em>Cx. perplexus</em></td>
<td>Tikri Kalan, Indri Block</td>
<td>2/135/0</td>
</tr>
</tbody>
</table>
at NICD. The other mosquito pools tested for virus infection were negative.

In the present study, MIR of Cx. tritaeniorhynchus in district Karnal of North India was found to be much higher (6.06) than that (0.84)\textsuperscript{1} from Cuddalore district of Tamil Nadu, South India. Although in an earlier communication JE virus antigen was detected in 30-day stored dry mosquitoes\textsuperscript{1} the present study supports the detection of virus antigen in long stored and unpreserved mosquitoes.

Detection of JE virus antigen in mosquitoes even after 20 months of dry storage at room temperature, in the present study is significantly important from the standpoint of JE surveillance, particularly in developing countries where facilities for storage and transportation of biological materials are inadequate.

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**REFERENCES**


