Entomological and serological investigation of Japanese encephalitis in endemic area of eastern Uttar Pradesh, India

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ABSTRACT

Background & objectives: Japanese encephalitis virus (JEV), a mosquito borne pathogen, is one of the major causes of viral encephalitis in eastern Uttar Pradesh, India. The objective of this work was to evaluate the entomological based virological surveillance of Japanese encephalitis (JE) in the highly endemic area of eastern Uttar Pradesh.

Methods: The study was carried out during September 2010 to March 2013 in Gorakhpur district of Uttar Pradesh. A total of 251 adult mosquito pools and 64 water samples containing larvae were collected from the District of Gorakhpur. Water pH, turbidity, and oxygen level were analyzed for vector breeding index (BI). In addition, 393 serum/cerebrospinal fluid (CSF) samples of acute encephalitis syndrome (AES) suspected cases were collected from the district hospital.

Results: The various Culex species found included, Cx. quinquefasciatus (26.83%), Cx. vishnui (22.29%), Cx. pseudovishnui (20.73%), Cx. tritaeniorynchus (12.71%), Cx. whitmorei (9.04%), and Cx. gelidus (8.25%). Highest minimum infection rate (MIR) was calculated for Cx. tritaeniorynchus (2.32), followed by Cx. vishnui (1.98) and Cx. pseudovishnui (0.71). All the larvae samples were negative for JEV. The mean number larvae of Cx. tritaeniorynchus and Cx. pseudovishnui was negatively correlated with pH (r = – 0.45 and r = – 0.63) and turbidity (r = – 0.30 and r = – 0.37). In contrast, positive correlation was observed in case of Cx. quinquefasciatus. A total of 41 clinical samples were found positive for JEV by IgM ELISA. The rainfall was significantly associated with Japanese encephalitis incidence and showed positive correlation to disease transmission (p = 0.02, r = 0.66).

Interpretation & conclusion: The findings showed the rapid dissemination of JEV within a population, facilitated by different species of Culex in the region. As JE is a vaccine-preventable disease, an immunization programme, an effective vector control strategy and application of standard hygiene practices in these endemic areas could result in a considerable reduction in morbidity and mortality due to JE.

Key words Culex mosquitoes; ecology; Japanese encephalitis; surveillance; vector

INTRODUCTION

Japanese encephalitis (JE) virus is the most important mosquito-borne flavivirus causing morbidity and mortality in humans1. In addition to JEV in India, there are several other endemic arboviruses like dengue, yellow fever, tick-borne encephalitis, West Nile and Rift Valley fever virus that cause acute encephalitis syndrome (AES)2. The JE case-fatality rate varies across geographical regions ranging from 10 to 30% among infected patients3-5. JE has been the leading cause of AES in Asia with 50,000 cases and 10,000 deaths reported annually6-8. In 1940, JE was first identified in China, and in 1949, it was identified in Korea and subsequently reported from India, in 19549-10. The following year, JEV strain was identified at Vellore, India in 195511. More recent outbreaks have been reported from Uttar Pradesh (3551 cases, 764 deaths) and Bihar (238 cases, 58 deaths) states in India and the western regions of Nepal (1540 cases, 259 deaths)12. Peak number of JE cases are reported from May to October in northern India, during and just after the rainy season that coincides with heavy rains and flooding13. Several ecological, biological and social factors influence larval and adult vector population and transmission of JE14. JE has been isolated from 16 species of mosquitoes belonging to the genera of Culex (10), Anopheles (3) and Mansonia (3)15. JE is a zoonotic disease where large water birds act as carriers, mosquitoes as vectors and domestic pigs are amplifying host, while humans are incidental hosts16. Climate is a major environmental factor influencing vector-borne disease epidemiology17. Outbreaks of JE in India are facilitated by several factors like temperature pattern, distribution of vector population, agriculture practices (wetland rice crop) swine rearing and their proximity to human populations17, 18-20. JE is a vaccine preventable disease. Due to the wide-
spread use of JE vaccine, JE cases have been reduced in China, Korea, and Japan\textsuperscript{21}. JEV vaccination (live attenuated Chinese vaccine SA-14-14-2) was started in Uttar Pradesh on 27 May 2007 by the Govt. of India. The vaccination programme covered 97% of targeted 10 million children between 1 and 15 yr-old, in the endemic Districts of Uttar Pradesh. JE vaccination has significantly contributed to the decline of JE positive cases from 35.88% as reported during 2005–06 to 7.28% during 2008–09 in the State of Uttar Pradesh, which contributes >80% of cases and deaths respectively in the country\textsuperscript{22}. Entomological surveillance was performed in association with serological findings of JE incidence in the highly endemic area of eastern Uttar Pradesh, India, to correlate the influence of environmental factors with vector breeding ecology and their involvement in disease transmission.

MATERIAL & METHODS

Study site

The study site, Gorakhpur district is well connected with all seven endemic districts of eastern Uttar Pradesh in India. In 2009, a total of 686 cases of AES were reported; out of which 76 cases and 19 deaths were confirmed due to JEV. Gorakhpur district lies between latitude 26° 46’ N and longitude 83° 22’ E. The district covers an area of 3483.8 km\textsuperscript{2}. It is surrounded by Maharajganj district to the north, Kushinagar and Deoria districts in the east, Ambedkar Nagar, Azamgarh, and Mau districts in the south, and Sant Kabir Nagar district in the west (Fig. 1). According to the 2011 census, the Gorakhpur district has a population of 4,440,895 with a population density of 1337 inhabitants/km\textsuperscript{2}, sex ratio of 950 females for every 1000 males and a literacy rate of 70.83%.

Entomological survey

Surveillance of adult mosquitoes: Adult mosquitoes were collected from September 2010 to March 2013 from different areas of Gorakhpur, such as paddy crops, wheat, millet, mustard, fodder plants, and other crops near houses using a battery operated, back packed aspirator\textsuperscript{23}. The selection of these sampling sites was based on the history of high JE incidences from different blocks, viz. Barhalganj, Bansgaon, Bhathat, Changanwla, Gagha, Gola Bazaar, Jungle Kaudia, Khajni and Pipraich. The time period for mosquito collection was between 0700 and 0900 hrs. Dead mosquitoes were discarded, and live ones were labeled and transported to the Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS) laboratory, Lucknow, where they were identified according to species identification keys developed by Reuben et al\textsuperscript{24} and stored at –20°C until tested.

Collection of larvae: A total of 11 larval breeding sites were surveyed in each nine blocks. The breeding habitats varied from human habitat water containing low lying flooded areas to animal hoof prints, ponds, puddle/borrow pits, paddy fields and stagnant water bodies. Water samples were collected by using 350 ml standard larval

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image1.png}
\caption{Map showing positive JEV cases and mosquito pools at different sample collection site of Gorakhpur district, Uttar Pradesh during 2010–13.}
\end{figure}
dipper from the same. Dips were taken gently with a 2–3-min pause, to allow the mosquito larvae to move freely in the air–water interface and henceforth a minimum of 5–10 dips were made from small and large water sources respectively. Larvae were identified after microscopic observation. Only Culex larvae were placed in glass/plastic vials containing water and stored at 4°C. Water analysis was performed using the Water Analyzer system (Type 371, Systronics India Ltd.) for pH, turbidity and dissolved oxygen level.

Collection of clinical specimens
Patients with clinical symptoms of high grade fever (≥39 °C) for <10 days with any two of the following symptoms: headache, vomiting, unconsciousness, convulsions, abnormal movements, stupor, delirium, altered sensorium, neck rigidity, presence of kernig’s sign; admitted to district hospital of Gorakhpur, were enrolled for the study. The 217 blood and 176 CSF samples were collected from suspected AES patients. Serum samples were separated from whole blood and aliquots stored at –80°C for further investigation.

Ethical approval
Ethical approval was obtained for human samples by the Ethical committee bioethics cell, SGPGIMS, Lucknow.

Processing of specimens
Adult and larval mosquitoes were pooled (20–25 mosquitoes/pool) by species, location and date of collection. Each pool was homogenized using a motor driven tissue grinder with 3 to 5 ml of Eagle’s minimal essential medium (MEM) media. The homogenate was subjected to centrifugation and supernatant was used for further screening.

RT-PCR
Viral RNA from adult mosquitoes and larvae were extracted using QIAamp viral RNA mini kit according to the manufacturer’s instructions (Qiagen). Quantitative real time polymerase chain reaction (qRT-PCR) for JEV (Rotor Gene 6000, Corbett Research, Australia) was done using Geno Sen’s JEV real time PCR kit (Genome diagnostic, India). The qRT-PCR were carried out by reverse transcription at 50°C for 15 min, activation at 95°C for 10 min, followed by 45 cycles at 95 °C for 15 sec, 50°C for 30 sec and 72°C for 15 sec.

Minimum infection rate (MIR)
The MIR was used to compare virus infection rates in Culex species. The MIR was calculated as number of viruses detected in mosquitoes by species/total number of mosquitoes of that species tested multiplied by 1000.

ELISA
Anti-JEV IgM and anti-dengue IgM antibodies in CSF and serum samples were tested by IgM capture ELISA (Panbio, Brisbane, Australia). The diluted sera and CSF were added to the assay plate, which contained anti-human IgM antibodies coated on the surface of the wells and incubated at 37°C for 60 min. Concurrently horseradish peroxidase (HRP)-Conjugated anti-JEV/dengue monoclonal antibody was added to diluted JEV/dengue virus type 1-4 antigens as per the kit manufacturer’s instructions. The absorbance was measured at 450 nm using an ELISA reader (Finstruments, Multiskan Model, Lab systems Finland, Type-347). The kit includes negative, positive and cut-off controls and results are based on a sample cut-off optical density ratio.

Meteorological data
Meteorological data including temperature (°C), rainfall (mm), and relative humidity (%) were obtained from the meteorological department, Government of India on regular basis during the study period.

Data surveillance
Data recorded from collected samples included each patient’s name, age, residential address along with their clinical diagnosis and environmental condition. All entomological, epidemiological and laboratory data were entered and analyzed using SPSS, version 20 and prism, version 5.01. Age, gender and environmental factor for JE distributions in endemic areas of Uttar Pradesh were determined.

Statistical analysis
Data were analysed using SPSS Version 20 (SPSS Inc., USA). The significant difference between number of larvae and area were assessed by Kruskal–Wallis test. Chi-square test and z test were performed to test the significant difference in MIR with the different areas and between species respectively. Spearman’s coefficient of co-relation was applied to check the correlation between number of dips and breeding index (BI). Bivariate correlation analysis was applied to check if the environmental factors play role in disease transmission. For breeding index analysis, a linear regression model was performed to determine if the rate of breeding index affects the rate of positive cases in JE endemic areas.
RESULTS

A total of 6784 mosquitoes were collected which comprised 1820 Cx. quinquefasciatus (26.83%), 1406 Cx. pseudovishnui (20.73%), 862 Cx. tritaeniorhynchus (12.71%), 624 Cx. whitmorei (9.20%) and 560 Cx. gelidus (8.25%). Out of these Cx. quinquefasciatus, Cx. vishnui and Cx. pseudovishnui were the most commonly collected mosquitoes from the surveyed area. The maximum number of Cx. tritaeniorhynchus was found in Charganwa (15.43%), Barhalganj (12.18%), and Gola Bazaar (11.95%). A total of six out of 251 pools were positive for JEV by real-time PCR which included three pools of Cx. vishnui, two of Cx. tritaeniorhynchus and one of Cx. pseudovishnui. Although, Cx. gelidus, Cx. whitmorei and Cx. quinquefasciatus did not show any positivity against JEV. Highest MIR was found in Cx. tritaeniorhynchus (2.32) and Cx. vishnui (1.98) as shown in Table 1. Distribution of positive mosquito pools in different areas was estimated. Two positive mosquito pools were found in Gola Bazaar. On the other hand, Bansgaon, Khajni, Charganwa and Pipraich accounted only for one positive mosquito pool in each site (Fig. 1). No significant difference was observed in MIR among different species of Culex (p = 0.89).

Larval and water analysis

A total of 350 dips were taken from 11 different sampling sites representing rural areas of study block. Out of 350, 117 were found positive for Culex larvae. Gola Bazaar (40.67%) showed maximum breeding index followed by Khajni (21.98%) and Bansgaon (21.75%) (Table 2). Each place differed significantly for the presence of Culex larvae (p = 0.01). Percentage of positive dips was found to be positively correlated with breeding index (p = 0.93) indicating increase in number of Culex species after every dip. A total of 64 larval pools were made from the most frequently collected species such as Culex vishnui (21), Cx. pseudovishnui (17) Cx. quinquefasciatus (14) and Cx. tritaeniorhynchus (12). The range of physico-chemical parameters of the collected water was: pH 6 to 8.13, turbidity 5.03–17.81 and 3.96 to 10.71 oxygen levels (Table 3). The mean number larvae of Cx. tritaeniorhynchus and Cx. pseudovishnui was negatively correlated with pH (r = −0.45 and r = −0.63) and turbidity (r = −0.30 and r = −0.37). The mean number larvae of Cx. quinquefasciatus was positively correlated with pH (r = 0.47) and turbidity (r = 0.22). All larvae samples were negative for JE virus by RT-PCR indicating that there is no transovarial infection of JE virus.

Table 1. Adult mosquito collection with minimum infection rate (MIR) at different block

<table>
<thead>
<tr>
<th>Species</th>
<th>Barhalganj</th>
<th>Bansgaon</th>
<th>Bhathat</th>
<th>Charganwa</th>
<th>Gagha Bazaar</th>
<th>Jungle Kaudia</th>
<th>Pipraich</th>
<th>Total no. of pools</th>
<th>Total Positive MIR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cx. vishnui</td>
<td>152</td>
<td>173</td>
<td>127</td>
<td>205</td>
<td>110</td>
<td>137</td>
<td>149</td>
<td>218</td>
<td>56</td>
</tr>
<tr>
<td>Cx. tritaeniorhynchus</td>
<td>105</td>
<td>78</td>
<td>93</td>
<td>133</td>
<td>90</td>
<td>103</td>
<td>87</td>
<td>94</td>
<td>2</td>
</tr>
<tr>
<td>Cx. gelidus</td>
<td>64</td>
<td>54</td>
<td>97</td>
<td>59</td>
<td>78</td>
<td>42</td>
<td>71</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Cx. whitmorei</td>
<td>79</td>
<td>76</td>
<td>73</td>
<td>57</td>
<td>73</td>
<td>70</td>
<td>71</td>
<td>61</td>
<td>0</td>
</tr>
<tr>
<td>Cx. pseudovishnui</td>
<td>195</td>
<td>176</td>
<td>153</td>
<td>112</td>
<td>177</td>
<td>141</td>
<td>157</td>
<td>159</td>
<td>52</td>
</tr>
<tr>
<td>Cx. quinquefasciatus</td>
<td>179</td>
<td>213</td>
<td>198</td>
<td>225</td>
<td>231</td>
<td>169</td>
<td>171</td>
<td>251</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2. Dipper index and breeding index of larvae in water sample by dip method

<table>
<thead>
<tr>
<th>Block/Site name</th>
<th>Total no. of dips</th>
<th>Positive dips</th>
<th>Dipper index (%)</th>
<th>Mean no. of larvae per dip</th>
<th>Breeding index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barhalganj</td>
<td>57</td>
<td>14</td>
<td>24.5</td>
<td>10.00 ± 3.136</td>
<td>9.12</td>
</tr>
<tr>
<td>Bansgaon</td>
<td>32</td>
<td>15</td>
<td>46.8</td>
<td>7.73 ± 3.453</td>
<td>21.75</td>
</tr>
<tr>
<td>Bhathat</td>
<td>44</td>
<td>12</td>
<td>27.2</td>
<td>10.67 ± 4.376</td>
<td>11.60</td>
</tr>
<tr>
<td>Charganwa</td>
<td>46</td>
<td>18</td>
<td>39.1</td>
<td>6.28 ± 3.045</td>
<td>17.15</td>
</tr>
<tr>
<td>Gagha</td>
<td>42</td>
<td>8</td>
<td>19</td>
<td>8.88 ± 3.563</td>
<td>1.69</td>
</tr>
<tr>
<td>Gola Bazaar</td>
<td>27</td>
<td>15</td>
<td>55.5</td>
<td>10.47 ± 3.226</td>
<td>40.67</td>
</tr>
<tr>
<td>Jungle Kaudia</td>
<td>30</td>
<td>7</td>
<td>23.3</td>
<td>9.00 ± 2.828</td>
<td>2.1</td>
</tr>
<tr>
<td>Khajni</td>
<td>39</td>
<td>17</td>
<td>43.5</td>
<td>7.82 ± 3.127</td>
<td>21.98</td>
</tr>
<tr>
<td>Pipraich</td>
<td>33</td>
<td>11</td>
<td>33.3</td>
<td>8.64 ± 3.042</td>
<td>8.64</td>
</tr>
</tbody>
</table>
Serological investigation
Sera collected from 217 patients during the study period, showed positivity for both JE and dengue viruses by IgM capture ELISA. A total of 22 (10.1%) serum samples were reactive against IgM antibody for JE, 4 (1.8%) were cross reactive for JE/dengue and 191 (88%) were negative for both. Similarly, 19 in 176 (8.7 %) CSF specimens were positive for anti-JE-IgM antibodies, confirming the involvement of JEV as an etiologic agent. However, only 41 (61% male and 39% female) cases were confirmed JEV positive. Although, JE cases were observed in all the age groups, the highest numbers of JE positive cases were observed in the age group of 0–10 yr. In total, 16 patients between age of >1–5 yr; and 10 from >5–10 yr were positive for JE (Fig. 2). Out of 41, the maximum number of JEV was reported from Charganwa (10), followed by Gola Bazaar (8), Bansgaon (6), Khajni (5), and Pipraich (4) (Fig. 1).

Correlation of environmental factor with JEV incidences
It was found that out of the three variables (rainfall, temperature, relative humidity) tested, only rainfall was significantly associated with JE incidences ($p = 0.02$, $r = 0.66$) as shown in Fig. 3.

Table 3. Mean of larvae on different water variables in various blocks

<table>
<thead>
<tr>
<th>Block/Site name</th>
<th>Barhalganj</th>
<th>Bansgaon</th>
<th>Bhathat</th>
<th>Charganwa</th>
<th>Gagha</th>
<th>Gola Bazaar</th>
<th>Jungle Kaudia</th>
<th>Khajni</th>
<th>Pipraich</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cx. vishnui</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.61 ± 0.13</td>
<td>7.42 ± 0.19</td>
<td>7.13 ± 0.03</td>
<td>7.80 ± 0.34</td>
<td>7.74 ± 0.05</td>
<td>7.43 ± 0.15</td>
<td>7.92 ± 0.10</td>
<td>6.84 ± 0.22</td>
<td>6.6 ± 0.19</td>
</tr>
<tr>
<td>Turbidity</td>
<td>5.03 ± 0.30</td>
<td>6 ± 0.91</td>
<td>9.06 ± 1.02</td>
<td>7 ± 1.3</td>
<td>12 ± 1.02</td>
<td>7 ± 0.91</td>
<td>8 ± 1.44</td>
<td>16 ± 2</td>
<td>8 ± 0.144</td>
</tr>
<tr>
<td>Dissolved DO</td>
<td>5.63 ± 0.01</td>
<td>4.23 ± 0.91</td>
<td>5.44 ± 0.40</td>
<td>7 ± 1.43</td>
<td>8 ± 0.33</td>
<td>4 ± 0.92</td>
<td>6.03 ± 0.33</td>
<td>7.45 ± 0.89</td>
<td>6.57 ± 1.14</td>
</tr>
<tr>
<td><strong>Cx. tritaeniorhynchus</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.14 ± 0.13</td>
<td>7.90 ± 0.77</td>
<td>6.25 ± 0.39</td>
<td>6.45 ± 0.87</td>
<td>7.45 ± 0.64</td>
<td>6.20 ± 0.48</td>
<td>7.34 ± 0.63</td>
<td>6.80 ± 1</td>
<td>7.44 ± 0.99</td>
</tr>
<tr>
<td>Turbidity</td>
<td>9.32 ± 1.02</td>
<td>5.71 ± 0.90</td>
<td>6.03 ± 0.79</td>
<td>14.87 ± 1</td>
<td>10.72 ± 1.40</td>
<td>9.26 ± 0.90</td>
<td>5.32 ± 0.57</td>
<td>7.63 ± 1.22</td>
<td>12.40 ± 1.72</td>
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<td>Dissolved DO</td>
<td>3.26 ± 0.47</td>
<td>5.78 ± 0.91</td>
<td>6.22 ± 0.85</td>
<td>4.92 ± 0.14</td>
<td>5.66 ± 0.63</td>
<td>7.45 ± 1</td>
<td>6.14 ± 0.63</td>
<td>7.44 ± 0.73</td>
<td>5.04 ± 0.44</td>
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<td><strong>Cx. quinquefasciatus</strong></td>
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<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.32 ± 0.05</td>
<td>6.31 ± 0.13</td>
<td>7.14 ± 0.41</td>
<td>6.83 ± 0.91</td>
<td>7.90 ± 0.47</td>
<td>8.13 ± 0.73</td>
<td>6.21 ± 0.80</td>
<td>6 ± 0.61</td>
<td>7.32 ± 0.37</td>
</tr>
<tr>
<td>Turbidity</td>
<td>5.14 ± 0.71</td>
<td>12 ± 1.21</td>
<td>9.41 ± 0.64</td>
<td>6.57 ± 0.83</td>
<td>7.41 ± 1.14</td>
<td>17.81 ± 1.37</td>
<td>9 ± 1.93</td>
<td>6.37 ± 0.63</td>
<td>14 ± 0.93</td>
</tr>
<tr>
<td>Dissolved DO</td>
<td>7.32 ± 0.40</td>
<td>5.09 ± 0.30</td>
<td>7.10 ± 0.71</td>
<td>6.32 ± 0.65</td>
<td>7.14 ± 0.94</td>
<td>5.32 ± 0.41</td>
<td>6.03 ± 0.91</td>
<td>5.43 ± 0.71</td>
<td>7.41 ± 0.64</td>
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<tr>
<td><strong>Cx. pseudovishnui</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>pH</td>
<td>6.22 ± 0.14</td>
<td>7.43 ± 0.91</td>
<td>6.43 ± 0.71</td>
<td>7.90 ± 0.83</td>
<td>6.52 ± 0.32</td>
<td>6.59 ± 0.14</td>
<td>5.98 ± 0.37</td>
<td>6.47 ± 0.44</td>
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<tr>
<td>Turbidity</td>
<td>5.46 ± 0.16</td>
<td>7.93 ± 0.44</td>
<td>12.11 ± 1.56</td>
<td>13.46 ± 1</td>
<td>8.19 ± 1.61</td>
<td>7.32 ± 0.57</td>
<td>6.14 ± 1.37</td>
<td>9.57 ± 1.40</td>
<td>12 ± 1.32</td>
</tr>
<tr>
<td>Dissolved DO</td>
<td>6.45 ± 0.19</td>
<td>7.43 ± 0.63</td>
<td>8.46 ± 0.71</td>
<td>4.34 ± 0.14</td>
<td>7.46 ± 1.32</td>
<td>5.43 ± 0.64</td>
<td>7 ± 0.37</td>
<td>5.83 ± 0.91</td>
<td>6.32 ± 0.47</td>
</tr>
</tbody>
</table>

Fig. 2: Age and gender-wise analysis of JE incidence in study area.

Fig. 3: Correlation and association of different environmental conditions with disease transmission— (a) Rainfall vs JE incidence; (b) Temperature vs JE incidence; and (c) Humidity vs JE incidence.
**Correlation of breeding index with JEV incidence**

Vector breeding index was found to be statistically significant with the positivity of JE cases in endemic areas \( (p = 0.035, r^2 = 0.491) \). However, Gola Bazaar, Khajni, Bansgaon and Charganwa represented higher breeding index indicating the high clinical positivity of JE cases in the same areas. In contrast, maximum number of *Cx. tritaeniorhynchus* and *Cx. vishnui* were found in those areas where number of ELISA positive cases was high (Fig. 1).

**DISCUSSION**

Based on the entomological surveys done in Gorakhpur region, species of *Culex* mosquitoes were found to be the most abundant species in all surveyed areas. This is because human habitat provided suitable breeding sites for the same\(^28\). In the present study, *Cx. quinquefasciatus* was the most abundant species found in urban, semi urban and rural areas. A wide range of *Cx. quinquefasciatus* has also been documented in India and other countries\(^29\). Being the cosmopolitan mosquito this species is found abundantly near households of urban, semi-urban and rural areas. In the present study the collection of *Cx. tritaeniorhynchus*, a primary vector for JEV, was found infrequent\(^30\). These differences in vector population might be attributed to changes in annual agricultural practices, improper usage of insecticides in the agriculture fields and variation in optimum humidity influencing the evaporation of water from breeding sites\(^16, 31\). In the study, highest MIR was recorded for *Cx. tritaeniorhynchus*. Similar to the study of Kanojia et al. most of the JE cases were found between August and November, following the decline of vector populations, particularly *Cx. tritaeniorhynchus*. Few environmental factors including rainfall, temperature and relative humidity, are also responsible for virus transmission\(^32\).

Result of our study represents that rainfall might be the important risk factor for JEV transmission. Present study reflects that the larvae of *Culex* species were abundantly found in Gola Bazaar, Khajani, Bansgaon and Charganwa exhibiting the high BI values. It was suggested that the water condition, *i.e.* pH, turbidity and dissolved oxygen of the breeding sites of larval species were found quite favourable for breeding of JE vectors in these areas. In the study, some study areas showed low BI value. Although, we didn’t quantified the presence of aquatic species that may cause harm to the larvae, but taking other studies into consideration, besides water conditions this may be one of the possible reasons for the low BI values in the studied areas\(^28\). Negative correlation of *Cx. tritaeniorhynchus* indicates, infestation rate of larvae decreased with increasing pH and turbidity. In contrast, positive correlation indicates increase in infestation rate of *Cx. quinquefasciatus* larvae with increasing pH and turbidity\(^28\).

On the basis of serological investigation; out of 393 cases 41 were found reactive against IgM antibody and had the history of illness for <10 days, indicative of an active immune response. Although, in some districts of eastern Uttar Pradesh like Gorakhpur, Kushinagar, Maharajganj, Basti, Deoria, and some adjoining areas of Bihar, the vaccine was provided through the National Vector Borne Disease Control Programme (NVBDC), cases are still reported annually\(^33-34\). In our study more males were affected than females with the predominant age group between 1 and 10 yr of age. The highest number of cases was observed in this age group possibly due to their low immunity\(^35\).

Moreover, the male individuals between the age of >15 and 20 yr usually take an active part in crop cultivation activities. The vector usually breeds in standing water in the cultivated rice paddies and grassy pools of water where the majority of this age group is exposed to vector populations. Maximum JE incidences were reported from Charganwa, Gola Bazaar, Bansgaon, Khajni and Pipraich which might be due to abundance of specific vectors, *Cx. tritaeniorhynchus* and *Cx. vishnui*. The overall report of low positivity of JEV was probably due to decline of primary vector in this region. A significant statistically positive correlation was noted in the present study between vector indexing and JE incidence. Thus, vector breeding index might be associated with the positivity of JE cases in this endemic region.

Viral diagnosis is tedious and expensive, and may not be possible for individual patients. Now, JE is considered the most common form of sporadic encephalitis in Uttar Pradesh. Lots of programmes were conducted by the Govt. of India for minimization of JE risk by controlling vector density. In rainy season, the incidence of both JEV and AES circulation was found to be increased. It was observed that some of the environmental conditions were statistically significant risk factors for transmission of JEV virus. A result of JEV negative in CSF and serum samples for ELISA also indicate the possibility of other pathogens that cause encephalitis, *e.g.* enteroviruses, such as coxsackie virus and herpes simplex virus in this area.

**CONCLUSION**

The current study has identified the potential or actual larval habitats of mosquitoes in the region and has
represented several correlations between vector density and their distribution pattern along with clinical association. JEV is emerging as a serious threat to human in endemic region of Uttar Pradesh. The abundance of JE vector and incidence of JEV cases in both rural and urban areas necessitate intensified surveillance and control of mosquito during high temperature and rainfall seasons, which may act as an effective strategy for controlling the burden of JE fever.

Conflict of interest
All authors declare to have no conflict of interest.

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REFERENCES

30. Kanoja PC, Shetty PS, Geevarghese G. A long-term study on vector abundance and seasonal prevalence in relation to the oc-
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