Japanese encephalitis (JE) is a major public health problem in India. To study the influence of climatic factors on JE, cases and the transmitting mosquito species were analysed during 2001 to 2006. To know the status of Japanese encephalitis virus activity in human population, sero-epidemiological studies were undertaken in villages of Kurnool district, Andhra Pradesh, India. Similarly, mosquitoes were sampled from study areas at bimonthly intervals during 2001 to 2006 and identified to species level. The collected mosquitoes were screened for JE virus by using an antigen-capture enzyme linked immunosorbent assay. Out of 2051 samples collected from the study areas, 156 (8%) sera samples were found to be positive for JE virus. The highest number of JE positive cases was observed in 2005 (14%), followed by 2003 (10.6%) and 2001 (9.1%). The seasonal pattern on occurrence of JE cases clustered among different seasons (that is, monsoon, winter and summer) showed that JE cases occurred in all seasons of the year. The vector, Culex tritaeniorhynchus per man hour density was correlated with (minimum and maximum temperature, p < 0.035 and p < 0.013), whereas Culex gelidus was positively correlated with rainfall (p < 0.05). JE transmission in temperate areas is dependent on climatic factors; however this study suggests that effects of weather variables such as rainfall, temperature and relative humidity might be responsible for increase of vector populations and also the JEV infection. Apart from these, the other factors like agricultural practices, virus amplifying hosts such as pigs and its density and virus reservoirs might also play a major role in the disease transmission in the study areas.

Keywords: Epidemiological survey, vector surveillance, Japanese encephalitis virus, climatic variables.

INTRODUCTION

Japanese encephalitis (JE) is one of the leading forms of viral encephalitis and is more prevalent in South Asia, Southeast Asia, East Asia and the Pacific (Solomon, 2006; Fischer et al., 2008). Mostly children and young adults are afflicted with JE in rural and suburban areas where rice cultivation and pig farming coexist (Fischer et al., 2010). In India, Japanese encephalitis virus (JEV) is an important pediatric public health problem (Kanojia et al., 2003; Srivastava et al., 2003) and was first reported in 1955 (Saxena and Dhole, 2008). Subsequently, JE epidemic have been reported in as many as 25 states/union territories of India (Kabilan et al., 2004). Recently in Uttar Pradesh, Bihar, and Andhra Pradesh, JE has emerged as a perennial public health problem (Arunachalam et al., 2009). An epidemic of JEV was reported in 2005 in Gorakhpur, Uttar Pradesh, India. It was the most severe epidemic in 3 decades; 5,737 persons were affected in 7 districts of Uttar Pradesh.
and 1,344 persons died (World Health Organization (WHO), 2006).

JE virus has been recovered from 19 mosquito species in different parts of India, and the prominent vectors are Culex tritaeniorynchus Giles and Culex vishnui Theobald (Murty et al., 2010). The JEV is mainly transmitted by the mosquito Cx. tritaeniorynchus in India, which prefers to breed in irrigated paddy fields (Upadhyayula et al., 2012). Wading ardeid water birds serve as reservoirs for this virus, but virus regularly spills over into pigs, members of the family of equidae and humans are generally thought to be dead-end hosts. The epidemiology of JE virus is not well understood and only little research has been done. This menace has now grown to be a serious infectious disease in developing countries like India. Hence, the present study is undertaken to investigate the influence of various climatic factors on JEV transmission in Kurnool district, Andhra Pradesh.

MATERIALS AND METHODS

Study area

Kurnool district of Andhra Pradesh, India was selected for the present study as highest number of JE cases were reported from this region since 1996. The area is located between 15.83°N, 78.05°E and its total population is 1,724,795. Most of the population is affianced in agricultural practices and are of low socioeconomic status. Patchy paddy fields and water loggings are commonly seen in and around Kurnool district that promotes the vector breeding. Domestic animals such as cattle, pigs and poultry commonly share the habitat with human population. This district is generally warm and humid during most parts of a year. The agricultural activities are at their peak during the Southwest monsoon which persists between June and October of the year. During the study period (2001 to 2006), the maximum temperature ranged from 30.3°C (December, 2005) to 42.8°C (May, 2003). Out of the 69 Primary Health Centers (PHCs) of Kurnool district, six areas namely Peddathumbalam, Nandanapalli, Nandikotkur, Gudur, Cherukulapadu and Kurnool have been selected for this study which showed highest number of JE cases since 1996. Data was collected on epidemiology, entomological and environmental parameters in index areas which are mentioned in the manuscript.

Epidemiological survey

In order to know the status of JE virus activity in human population, sero-epidemiological studies were undertaken in the study areas of Kurnool district. The blood samples were collected from the school children (6 to 10 years old) through finger prick method. The sera of school children were examined by haemagglutination inhibition (HI) test following protocol of Clarke and Casals (Arunachalam et al., 2009) for identifying the flavivirus antibodies (JE/West Nile/Dengue).

Mosquito collection

Mosquitoes were sampled from identified villages at bimonthly intervals during 2001 to 2006 and identified to species level (Arunachalam et al., 2009). Both blood engorged and unfed adult mosquitoes, resting on bushes and thatched roofs of cattle sheds and human dwellings were trapped from different parts of the village. Fully fed mosquitoes were held for 24 to 48 h for digestion of blood meals. Later the captured mosquitoes were segregated into different pools (50 mosquitoes per pool) and were screened for JEV infectivity by using an antigen-capture enzyme linked immunosorbent assay (ELISA) for the initial screening of flavivirus and inoculation of Toxorhynchites splendens (Wiedemann) combined with an indirect immunofluorescence assay (Toxo-IFA) which was performed to confirm infection with JEV. Virus infection rate in mosquitoes was expressed as minimum infection rate (MIR) per 1000 females tested (Arunachalam et al., 2009).

\[
MIR = \frac{\text{No. of positive pools}}{\text{Total no. of specimens tested}} \times 1000
\]

Meteorological data

Besides epidemiological and entomological studies, data on other parameters like maximum and minimum temperature, rainfall, relative humidity and wind speed were collected month wise from meteorological department, Hyderabad, Government of India during study periods.

Ethics statement

The study received ethical clearance from the Ethical Committee which was constituted in our institutes (Indian Institute of Chemical Technology & Centre for Research in Medical Entomology) affiliated to Ministry of Science & Technology and Ministry of Health & Family Welfare, Govt. of India. This ethical committee has approved to carry out the research work. The consent of the subjects who provided the blood sample was minors. Hence written consent was obtained from the parents/guardians before the commencement of epidemiological survey. Similarly, entomological survey was also conducted in private land/private residences after obtaining the written consent from the respondents.

Statistical analysis

Data analysis was done with the SYSTAT statistical package. Most of the JE cases occurred in July to September (monsoon period). In order to understand the disease transmission dynamics, the samples were also collected during winter (November to January) and summer (March to May) seasons. The incidences of JE, mosquito density and MIR were correlated with climatic factors by using Spearman’s rank correlation method.

RESULTS

Out of 2,051 samples collected from the study areas (Figure 1), only 156 (8%) sera samples were found to be positive for JE virus by HI method. The highest number of JE positive cases was observed in 2005 (14%), followed by 2003 (10.6%) and 2001 (9.1%). Between the years 2002 and 2003, an increasing trend of JE cases was observed but the year 2004 had less number of cases.
reported (Figure 2). The percentage of JE positive cases are found to be weak to moderate correlation with minimum infection rate (MIR) of *Cx. tritaeniorhynchus* \((r = 0.26, p < 0.6)\) and *Cx. gelidus* \((r = 0.36, p < 0.5)\).

To understand the disease transmission dynamics, sero-epidemiological survey was also carried out during different climatic seasons of the year. From the study, it is noticed that number of cases were mostly recorded during the monsoon period (from July and attains highest number in September). Among the seasonal data on JE cases, highest number of positive cases was recorded during summer season of the year 2005 (Figure 3). Weak to moderate correlation was observed in the number of JE positive cases while comparing with per man hour (PMH) density of *Cx. tritaeniorhynchus* \((r = -0.35, p < 0.4)\) and *Cx. gelidus* \((r = -0.43, p < 0.3)\). Similarly, correlation analysis has been done with weather variables and incidence of JE cases during the study period (Figure 4). The results in Table 1 indicate that the maximum, minimum temperature, relative humidity and rainfall were
found weak to moderate correlation on the number of JE cases.

In Table 2, the correlation coefficient was evaluated between climatic variables and vector density of C.
Table 1. Correlation between climatic variables and JE cases in Kurnool district of Andhra Pradesh, India.

<table>
<thead>
<tr>
<th>Climatic variables</th>
<th>r-Value</th>
<th>p-Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainfall</td>
<td>-0.152</td>
<td>0.745</td>
<td>-0.812-0.679</td>
</tr>
<tr>
<td>Maximum temperature</td>
<td>0.372</td>
<td>0.411</td>
<td>-0.529-0.879</td>
</tr>
<tr>
<td>Minimum temperature</td>
<td>0.225</td>
<td>0.627</td>
<td>-0.636-0.836</td>
</tr>
<tr>
<td>Relative humidity at 08.30 h</td>
<td>-0.381</td>
<td>0.399</td>
<td>-0.881-0.522</td>
</tr>
<tr>
<td>Relative humidity at 17.30 h</td>
<td>-0.387</td>
<td>0.391</td>
<td>-0.883-0.517</td>
</tr>
</tbody>
</table>

Table 2. Correlation between climatic variables and per man hour density of *C. tritaeniorhynchus* and *C. gelidus*.

<table>
<thead>
<tr>
<th>Climatic variables</th>
<th><em>C. tritaeniorhynchus</em></th>
<th><em>C. gelidus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r-Value</td>
<td>p-Value</td>
</tr>
<tr>
<td>Rainfall</td>
<td>-0.317</td>
<td>0.489</td>
</tr>
<tr>
<td>Maximum temperature</td>
<td>-0.789</td>
<td>0.035*</td>
</tr>
<tr>
<td>Minimum temperature</td>
<td>-0.861</td>
<td>0.013*</td>
</tr>
<tr>
<td>Relative humidity at 08.30 h</td>
<td>0.684</td>
<td>0.09</td>
</tr>
<tr>
<td>Relative humidity at 17.30 h</td>
<td>0.346</td>
<td>0.447</td>
</tr>
</tbody>
</table>

*P<0.05.

Figure 5. Seasonal prevalence of *C. tritaeniorhynchus* and *C. gelidus* with temperature, rainfall and relative humidity in Kurnool district of Andhra Pradesh.

*tritaeniorhynchus* and *C. gelidus*. In *C. tritaeniorhynchus* PMH density found correlation exists with temperature (p < 0.035 and p < 0.013) (Figure 5), whereas *C. gelidus* was positively correlated with rainfall (p < 0.05). The MIR showed moderate correlation with *C. tritaeniorhynchus* (r = 0.338, p < 0.289) and *C. gelidus* (r = 0.373, p < 0.268) PMH density. Similarly, *C. tritaeniorhynchus* MIR were correlated with weather variables and the MIR values had
shown that weak to moderate correlation exist, whereas C. gelidus showed strong correlation significance with maximum temperature (p < 0.074) and relative humidity (p < 0.065) (Table 3).

**DISCUSSION**

JE is a major public health problem in India and mainly affects the children and young adults (Swami et al., 2008). It is estimated that 3 billion people live in Japanese encephalitis virus (JEV) endemic regions and the disease incidence are about 50,000 cases and 10,000 deaths annually (Fischer et al., 2008). From this study, out of 2051 samples collected, 156 (8%) sera samples were found positive for JEV. The year-wise JE positives cases during the study period (2001 to 2006) are presented in Figure 2 which shows that the JEV infection in this area is in the immediate past. To understand the prevalence of JE cases among the different seasons of a year, it was noticed that highest number of JE cases were mostly reported in JEV transmission period that is, monsoon period (July to September) (Figure 3). Higher number of cases during this period may be due to the moderate to high rainfall and cultivation of paddy fields (preferred breeding place of Culex). These suitable conditions help the mosquitoes to breed and transmit the disease at higher rate than the rest of the months of a year (Sarkar et al., 2012). However, JE cases were also reported during summer seasons of a year 2004 and 2005 (no data available for year 2001 to 2003 for summer season).

To observe the disease transmission dynamics during summer season (that is, March to May) of the years, it is noticed that higher numbers of JE positive cases were recorded (1.4% in 2004 and 19.3% in 2005). Occurrence of JE cases during summer seasons may be due to high precipitation, suitable temperature for JEV propagation in mosquitoes. In contrast, heavy rainfall (April, 64 mm and May, 113.7 mm) was recorded during summer season of 2005 and coincidentally highest number of JE cases were reported (19.3%) during this period. Similar kind of reports was also observed in Eastern China, where JE cases were reported in summer season (Upadhyayula et al., 2012). Higher number of cases may be due to creation of many breeding sources for Culex mosquito species in paddy fields and might be responsible for the increased risk of JE cases (Khan et al., 1996).

Sero-epidemiological surveys were also conducted in winter season (that is, November, 2005 to January, 2006) where (7.6%) of JE cases were reported. JE cases during winter season may be due to second phase cultivation period of paddy where there were sufficient numbers of mosquito breeding places. Similar kinds of data were also reported on JE cases during winter season (Sarkar et al., 2012; Khan et al., 1996). Based on these study reports, JE tends to be endemic, and cases occur sporadically throughout the year with a peak after the start of the rainy season. Hence, it is assumed that climate variability has a direct influence on JE cases (Githeko et al., 2000). Temperature (22 to 34°C) and relative humidity (42.7 to 69.6%) are ambient conditions to facilitate the higher mosquito density as well as JE virus replication and occurrence of JE cases (Murty et al., 2010).

In the present study, it is noticed that minimum and maximum temperature were found to be positively correlated with number of JE cases but negative correlation with rainfall and relative humidity (Table 1). It is also noticed that the average rainfall during the study period was 70.5 mm and relative humidity was between (45.6 to 70.1%). Even though, with this low rainfall and low relative humidity, occurrence of many JE cases were noticed, and this may be due to the availability of sufficient numbers of breeding places for mosquitoes and suitable temperature for development and transmission of JE virus.

C. tritaeniorhynchus has also been incriminated as a major vector of JE in India as well as in many countries of Southeast Asia (Murty et al., 2010). In the present study also we have recorded that JEV was mainly transmitted by the C. tritaeniorhynchus and C. gelidus mosquito species. Their abundance was mainly noticed during the paddy cultivation periods. In Kurnool district, generally two rice crops are grown in a year (from July to November and December to April). As a result, C. tritaeniorhynchus showed increase in abundance during September and January, corresponding to rice crop

### Table 3. Correlation between climatic variables and minimum infection rate of C. tritaeniorhynchus and C. gelidus.

<table>
<thead>
<tr>
<th>Climatic variables</th>
<th>C. tritaeniorhynchus MIR</th>
<th>C. gelidus MIR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r-Value</td>
<td>p-Value</td>
</tr>
<tr>
<td>Rainfall</td>
<td>0.227</td>
<td>0.714</td>
</tr>
<tr>
<td>Maximum temperature</td>
<td>-0.095</td>
<td>0.879</td>
</tr>
<tr>
<td>Minimum temperature</td>
<td>0.327</td>
<td>0.591</td>
</tr>
<tr>
<td>Relative humidity at 08.30 h</td>
<td>0.362</td>
<td>0.55</td>
</tr>
<tr>
<td>Relative humidity at 17.30 h</td>
<td>0.438</td>
<td>0.46</td>
</tr>
</tbody>
</table>
(Figure 3). Likewise, the vector \textit{C. gelidus} density was also found high in JE transmission period and this vector is normally bred in the stagnated water bodies. Most of the researchers have reported that the vector density is associated with the JE cases but in the present study, the vector density that is, PMH of \textit{C. tritaeniorhynchus} and \textit{C. gelidus} showed negative correlation with number of JE positive cases when compared with the various climatic variables (rainfall and temperature) (Table 2 and Figure 5). Similar type of reports was found where dengue incidence was negatively associated with rainfall (Thammapalo et al., 2005).

The JEV infection rates in female \textit{C. tritaeniorhynchus} and \textit{C. gelidus} mosquitoes varied largely. To understand the transmission dynamics of virus by vectors and their spatial variations within a JE endemic district of Andhra Pradesh, this study was undertaken, covering the whole year. Vector susceptibility in different years showed \textit{C. tritaeniorhynchus} MIR 1.9% with 10.6% JE cases in 2003 and 1.5% with 14% JE cases in 2005. Similarly in 2005, higher MIR was reported in \textit{C. gelidus} (4.2%) and correspondingly 14% of JE cases were reported in the study area. Monthwise data shows that the higher MIR values were reported in the September to November and extended up to December and was least during summer season of year. This suggests that variations in infection during these different seasons are mainly responsible for transmission of JEV to humans. Thus, temporal changes might have greatly impacted the efficiency of arboviral transmission in nature, which would have significant epidemiological importance.

In our study, lower MIR in both \textit{C. tritaeniorhynchus} and \textit{C. gelidus} mosquitoes have been reported, similar results of lower infection rates were also observed by earlier researchers (Samuel et al., 2010). This may be due to the low virus titer and quantity in the blood meal or due to the presence of several anatomical or physiological barriers (Bi et al., 2003). However, it is also suggested that the variations in the number of JE cases and transmission of JEV may be due to human age distribution, type of mosquito’s species as well as on the influence of various climatic factors for the growth, development and propagation of both virus and vectors. The climate variables such as temperature, rainfall and relative humidity might have a significant impact on the transmission of the diseases (Upadhyayula et al., 2012).

Correlation analysis in this study showed that MIR of both \textit{C. tritaeniorhynchus} and \textit{C. gelidus} mosquito species were positively correlated to percentage of JE positive cases. Similarly, correlation of MIR with climatic factors showed positive relation with rainfall, relative humidity and negative relation with temperature (Table 3). The MIR obtained during the study period 2001 to 2005 showed correlation with the abundance of the vectors which ranged for \textit{C. tritaeniorhynchus} (65 to 490.16 PMH) and \textit{C. gelidus} (6 to 157.33 PMH).

This longitudinal study reveals a steady increase of JE cases from 2001 to 2005, indicating a possible public health threat in the near future. The incidence of JE infection is relatively high during monsoon period and it strongly depends on rainfall, humidity and temperature as well as the paddy cultivation. Effects of climate change on rainfall, temperature and other climatic variables may increase the vector populations and risk of JEV infection, especially in temperate regions like India. Apart from these factors, availability of high mosquito abundance, virus reservoirs, virus infected mosquitoes, development of resistance to effective insecticides, rapid globalization, population explosion and global climate change have also influenced the endemcity of this disease (Karuanaratnae and Hemingway, 2000; Ghosh and Basu, 2009). As of now, no disease surveillance studies have been carried out in this region, and this study is to make an attempt to understand the disease scenario and vector dynamics in relation with weather variables.

**ACKNOWLEDGEMENTS**

Authors are grateful to Directors, CSIR-Indian Institute of Chemical Technology, Hyderabad and Centre for Research in Medical Entomology (ICMR), Madurai for their continuous support and encouragement.

**REFERENCES**


Karuanaratnae SHPP, Hemingway J (2000). Insecticide resistance


