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Impact of weather variables on mosquitoes infected with Japanese encephalitis virus in Kurnool district, Andhra Pradesh

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ABSTRACT

Objective: To assess the virus infection in mosquitoes during different seasons and correlated with various climatic factors. **Methods:** The field collected vectors were screened for Japanese encephalitis (JE) virus after dessication using ELISA method. Most of the positive pools were recorded from *Culex tritaeniorhynchus* (*Cx. tritaeniorhynchus*) and *Culex. gelidus* (*Cx. gelidus*) during JE transmission season (winter) and some positive pools were also reported during non JE transmission periods (*i.e.* summer and rainy seasons). **Results:** The minimum infection rates (MIR) of 1.75 from *Cx. tritaeniorhynchus* and 0.17 from *Cx. gelidus* has been reported in the year 2002 at the beginning of the study and the values were found nil at the end of the study (2006) from the study areas of Kurnool district. **Conclusions:** From this study it is noted that MIR of *Cx. gelidus* and *Cx. tritaeniorhynchus* were modulated by various meteorological parameters. The mosquito vector abundance increases after the monsoon period (winter) and lowest in dry season (summer). Similarly, MIR fluctuated between seasons with higher MIR recorded after monsoon period and lower in the rest of season. Impact of these metrological parameters in JE virus infected mosquitoes is discussed in this paper.

1. Introduction

Japanese encephalitis (JE) is endemic mainly to rural areas with rice fields domiciling the breeding zones for vectors. It is estimated that 1.9 billion people are currently living in rural JE-prone areas of the world. Among them, 220 million people live in proximity to rice irrigation schemes[1]. The paddy breeding *Culex tritaeniorhynchus* (*Cx. tritaeniorhynchus*) and members of the closely related *Culex vishnui* (*Cx. vishnui*) group are principal vector of JE, this mosquito feeds preferentially on large domestic animals and birds and infrequently on humans. Pigs serve as amplifying hosts. In contrast, humans are dead end hosts because they experience short duration and low level viremia[2]. Over the past decade, there has been a pattern of steadily enlarging recurrent seasonal outbreaks in various parts of South East Asian countries (Vietnam, Thailand, Nepal, India, Philippines, Indonesia, and Australia)[3].

Japanese encephalitis virus (JEV) is a major public health problem worldwide and its outbreaks are noticed frequently in South East Asian countries[4]. In India, JEV was first recorded during 1955 in the state of Tamilnadu but in due course of time, this disease has spread to 25 states/union territories at varying degree of intensity[5,6]. JEV is transmitted mostly by *Cx. tritaeniorhynchus* and *Cx. gelidus* in India[7]. Many surveillance studies have been conducted elsewhere to identify the factors for outbreak of JEV and it is understood that, JE transmission is mainly facilitated by two important factors namely, global climate change and the modulation of agriculture[8]. In one of the reports it is mentioned that, impact of climate variability on the transmission of Japanese encephalitis virus is principally

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influenced by temperature and precipitation, for the growth of the mosquitoes and the JEV[9]. There are also few studies conducted on the influence of Rainfall, temperature and relative humidity on the density of mosquitoes[10,11] and the transmission of malaria, hemorrhagic fever with renal syndrome, dengue fever, and Ross River virus infections[12–15]. However, little research has been done on Japanese encephalitis, which is a serious infectious disease in developing countries like India. Hence, the intention of the study is to understand better the JEV cycle and its seasonal transmission dynamics due to effect of various climatic factors also the occurrence of minimum infection rate (MIR) of JEV in mosquitoes in Kurnool district of Andhra Pradesh.

2. Materials and methods

2.1. Study area

The study was carried out in Kurnool district of Andhra Pradesh (Figure 1). This district is located 15.83°N, 78.05°E and has warm and humid climate throughout the year with three major seasons *i.e.* summer (March–June), rainy (July–October) and winter (November–February). The majority of people depend upon agricultural work and rearing of domestic animals such as cattle and pigs. Most of the agricultural activity is conducted during the Southwest monsoon; between July and October. Among the 615 villages in 69 Primary Health Centres (PHCs) of Kurnool district, five villages (Peddathumbalam, Nandanapalli, Nandikotkur, Gudur, Cherukulapadu villages) were selected for the study based on past JE incidence. In addition the Kurnool urban region/city was also included.

Figure 1. Map of Kurnool district showing location of study areas.

2.2. Mosquito collection

The mosquito surveillance studies were conducted between July 2002 and May 2006. Mosquitoes were collected at

Table 1

Estimates of JEV infection rates of *Cx. tritaeniorhynchus* and *Cx. gelidus* mosquito pools collected during different seasons from 2002–2006 in Kurnool district of Andhra Pradesh.

Year	Season	*Cx. tritaeniorhynchus*				*Cx. gelidus*			
		No. of pools	No. of +ve pools	MIR/ 1 000	Confidence interval (Lower, upper)	No. of pools	No. of +ve pools	MIR/ 1 000	Confidence interval (Lower, upper)
2002	Summer (March–June)	12	0	0.00	0.06, 0.64	0	0	0.00	0.01, 0.70
	Rain (July–Oct)	71	0	0.00		13	0	0.00	
	Winter (Nov–Feb)	172	3	1.75		126	1	0.17	
	Total	255	3	1.75		139	1	0.17	
2003	Summer (March–June)	6	0	0.00	0.14, 0.70	10	0	0.00	0.12, 0.87
	Rain (July–Oct)	117	0	0.00		121	3	9.17	
	Winter (Nov–Feb)	235	6	3.43		92	1	333.33	
	Total	358	6	3.43		223	4	342.50	
2004	Summer (March–June)	14	0	0.00	0.01, 0.78	80	4	3.29	0.26, 1.56
	Rain (July–Oct)	29	1	1.53		29	1	0.84	
	Winter (Nov–Feb)	82	0	0.00		35	0	0.00	
	Total	125	1	1.53		144	5	4.13	
2005	Summer (March–June)	12	1	3.18	0.50, 1.58	3	0	0.00	0.24, 4.32
	Rain (July–Oct)	45	2	2.03		23	1	2.43	
	Winter (Nov–Feb)	125	6	0.85		5	1	23.26	
	Total	182	9	6.06		31	2	25.69	
2006	Summer (March–June)	49	0	0.00	0.00, 1.42	9	0	0.00	0.00, 1.40
	Rain (July–Oct)	3	0	0.00		44	0	0.00	
	Total	52	0	0.00		53	0	0.00	

bimonthly intervals from (resting on vegetation, bushes and thatched roofs of cattle sheds and human dwellings) using mouth aspirator and light–traps. The collected mosquitoes were then subsequently transported to the field laboratory for further processing. Only female mosquitoes were utilized in this study. Fully–fed mosquitoes were held for 24–48 h for digestion of blood meals, anesthetized with ether, identified to species level using the key developed by Reuben[16] and stored on ice after separating into pools comprising of 50 each, and these samples were further used for virus isolation.

2.3. Virus detection

Female mosquitoes were tested in single–species pools of 3–50 for JE virus by using an antigen–capture enzyme linked immunosorbent assay (ELISA) for the initial screening for flavivirus and inoculation of *Toxorhynchites splendens* (Wiedemann) combined with an indirect immunofluorescence assay (Toxo–IFA) was performed to confirm infection with JEV[17].

MIR = No. of positive pools/Total no. of specimens tested × 1 000.

Virus infection rate was expressed as maximum likelihood estimate with 95% confidence intervals by using the software (http://www.cdc.gov/ncidod/dvbid/westnile/software.htm).

2.4. Meteorological data

Month wise meteorological data of Kurnool district (on maximum and minimum temperature, Rainfall, relative humidity and wind speed) were compiled from the Meteorological Department, Hyderabad, Govt. of India during the study period.

3. Results

A total number of 61 848 mosquitoes were collected over the entirety of the project. A total of 972 pools (30 048 specimen) of *Cx. tritaeniorhynchus* and 590 pools (19 988 specimens) of *Cx. gelidus* were screened for JEV activity. Similarly other species *i.e.* two pools (11 specimens) of *Cx. pseudovishnui* and 14 pools (483 specimens) of *Anopheles subpictus* were also analysed for flavivirus infection but JEV was only found in *Cx. tritaeniorhynchus* and *Cx. gelidus*. 19 pools of *Cx. tritaeniorhynchus* and 12 pools of *Cx. gelidus* were found to be positive for JEV antigen by ELISA method. The remaining collected mosquitoes (11 318 male and female) were not subjected for the study as they were the non JE vectors belonging to different species. See Table 1 for inter–annual JEV infection rates of *Cx. tritaeniorhynchus* and *Cx. gelidus*.

Most of the positive pools were recorded from *Cx. tritaeniorhynchus* and *Cx. gelidus* during JE transmission season (winter) and some positive pools were also reported during non JE transmission periods (*i.e.* summer and rainy seasons). The highest number of positive pools in *Cx. tritaeniorhynchus* was six reported during the year 2003 in JE transmission period (winter), similarly four pools of *Cx. gelidus* were found positive in 2004 during summer season. The MIR of 1.75 from *Cx. tritaeniorhynchus* and 0.17 from *Cx. gelidus* has been reported in the year 2002 at the beginning of the study and the values were found nil at the end of the study (2006) from the study areas of Kurnool district (Table 1) (Figure 2). Pearson correlation coefficient was evaluated to assess the relationship between MIR and the maximum, minimum temperature, rainfall, relative humidity and wind speed. Most of the variables have shown negative correlation with MIR for both *Cx. tritaeniorhynchus*, *Cx. gelidus* species (Table 2).

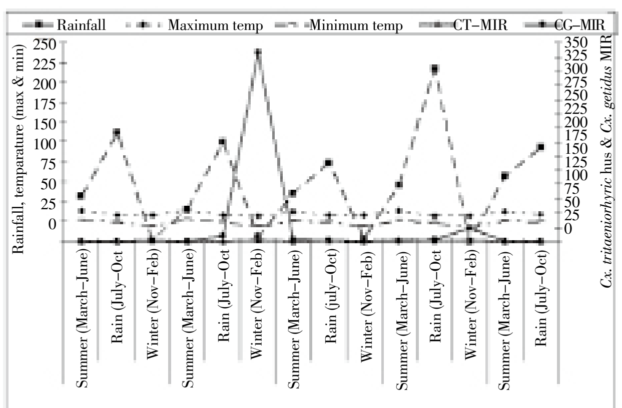

Figure 2. Climatic factors and JEV infection of *Cx. tritaeniorhynchus* and *Cx. gelidus* in Kurnool district of Andhra Pradesh.
CT: *Cx. tritaeniorphyndus*, CG: *Cx. gelidus*.

Table 2
Pearson correlation coefficients between climatic variables and MIR of *Cx. tritaeniorhynchus* and *Cx. gelidus* mosquitoes collected during 2002–2006.

Climatic variable	*Cx. tritaeniorhynchus*		*Cx. gelidus*	
	MIR	*P*–value	*MIR*	*P*–value
Rainfall	– 0.085	0.38	– 0.319	0.13
Maximum temperature	– 0.257	0.18	– 0.304	0.14
Minimum temperature	– 0.352	0.10	– 0.458	0.04
Relative humidity (08.30 h)	0.113	0.35	0.033	0.45
Relative humidity (17.30 h)	– 0.015	0.47	– 0.170	0.28
Average wind speed	– 0.294	0.15	– 0.427	0.06

Table 3

Season wise correlation coefficients (*r*), *P*-values between climatic variables of *Cx. tritaeniorhynchus* from 2002-2006.

Season	Statistics	Rainfall	Max temp	Min temp	RH-08.30	RH-17.30	Wind speed	*CI*(Lower, Upper)	MIR
Rainy	*r*-Value	0.53	- 0.84	- 0.76	0.83	0.74	- 0.53	0.06, 0.61	0.712
	P-Value	0.20	0.04	0.08	0.04	0.09	0.20		
	Mean	138.95	33.20	23.96	75.88	56.85	4.31		
Winter	*r*-Value	0.31	0.11	- 0.16	- 0.05	0.09	0.16	0.29, 0.80	1.508
	P-Value	0.32	0.44	0.41	0.47	0.45	0.41		
	Mean	2.67	32.21	18.74	71.31	37.06	1.37		
Summer	*r*-Value	0.55	- 0.13	- 0.19	- 0.55	- 0.65	0.30	0.01, 1.04	0.636
	P-Value	0.27	0.45	0.42	0.27	0.22	0.38		
	Mean	61.26	38.55	26.25	61.55	34.20	4.10		

Table 4

Season wise correlation coefficients (*r*), *P*-values between climatic variables of *Cx. gelidus* from 2002-2006.

Season	Statistics	Rainfall	Max temp	Min temp	RH-08.30	RH-17.30	Wind speed	*CI*(Lower, Upper)	MIR
Rainy	*r*-Value	0.03	- 0.20	- 0.15	0.18	0.15	- 0.68	0.16, 0.97	2.480
	P-Value	0.48	0.37	0.40	0.38	0.40	0.10		
	Mean	138.95	33.20	23.96	75.88	56.85	4.31		
Winter	*r*-Value	0.80	- 0.35	- 0.31	- 0.50	- 0.85	0.27	0.06, 0.63	89.190
	P-Value	0.09	0.32	0.34	0.24	0.08	0.36		
	Mean	2.67	32.21	18.74	71.31	37.06	1.37		
Summer	*r*-Value	- 0.08	- 0.35	- 0.51	0.04	0.25	- 0.11	0.26, 1.91	0.658
	P-Value	0.45	0.28	0.18	0.47	0.34	0.43		
	Mean	61.26	38.55	26.25	61.55	34.20	4.10		

4. Discussion

Seasonal rains are followed by increase in mosquito population and transmission. The seasonal distribution of vector mosquitoes varies in time and space depending upon environmental conditions and availability of breeding habitats. This is probably related to the growth and development of vectors, and the propagation of the virus in mosquitoes. *Cx. tritaeniorhynchus* populations were more abundant during the monsoon and immediate post monsoon seasons due to the wetness saturation in paddy fields and rainwater pools. *Cx. tritaeniorhynchus* population is strongly affected by the availability of suitable breeding habitats. *Cx. gelidus* was the predominant species, and no seasonal pattern on population density[18] had been reported. There was a distinct seasonal distribution of JE cases in the study period. In turn meteorological parameters induced influences in case fluctuations. Also there lies a great difference in the infection rates of the virus between the mosquitoes and pigs at various temperatures[19]. In the present study, we correlate minimum infection rate of *Cx. tritaeniorhynchus* and *Cx. gelidus* with meteorological parameters and hence to analyze the significance of variations in MIR values through different seasons of the year. These findings on vector competence could aid in clarifying the transmission pattern of JEV in different parts of India[20,21].

Climate variability has a direct influence on the epidemiology of vector-borne diseases[22]. Mosquito infection rates (MIR) display considerable geographical and temporal variation, reflecting the complex interaction of climatic patterns, agricultural practices, mosquito population dynamics, and the presence of susceptible amplifying hosts. In this study, vector susceptibility in different seasons showed a higher MIR of 1.50 during the rainy (July-October) and winter (November-February) seasons which coincided with peak transmission season of JE, and a lower MIR of 0.636 arithmetic mean during summer (March-June) for *Cx. tritaeniorhynchus*. Similarly the other JE vector, *Cx. gelidus* provided in rainy period, an arithmetic mean of 2.488, 89.19 in winter and lower MIR of 0.65 was observed during non-transmission (summer) period of JE. This suggests that the intensity of infection rate varies in different seasons it also either increases/decreases the rate of endemicity of the disease during the corresponding period.

The results show that variation of MIR peaked during the JE transmission period (winter). Apparently, MIR of *Cx. gelidus* and *Cx. tritaeniorhynchus* are influenced by various meteorological parameters, from this study it was noticed that the infection rate varied significantly with temperature when compared with other climatic conditions. These environmental parameters may be responsible for increase in multiplication of virus inside the reservoir hosts. Similar kind of results has also been reported for transmission of JE in urban areas of Jinan city of China. It is also noticed that, when the temperature goes beyond 32 ℃, there is a possibility of reduction in virus multiplication inside the host and therefore less infection rate has been remarked during summer season of a year[23]. Earlier reports also state that, extrinsic incubation period and viral development rate can be shortened at higher temperatures[24]. Minimum temperature comes out to be most critical factor in many regions as the threshold factor for mosquito survival, in sustaining the population density and eventually regulates the rate of viral transmission[25,26].

Cx. tritaeniorhynchus is largely responsible for transmission of JE in Kurnool district of Andhra Pradesh, India, although other species such as *Cx. gelidus* have been identified in urban areas of our study area. In this study, mosquito vector abundance increased after the monsoon period (winter) and lowest in dry season (summer). Similarly, MIR fluctuated between seasons higher MIR was recorded after monsoon period and lower in the rest of season. Similar seasonality and inter-annual variation in arbo-virus incidence was reported in other parts of the world also[27,28]. This study therefore improves the understanding of host-pathogen interactions. Screening JEV infection in mosquitoes collected in study areas serves in determining and categorizing the level of risk to human infections. In due course, such studies will help to develop disease predictive models and early warning system based on various parameters and in making decisions on public health prevention program such as vector control, environmental intervention and personal protection.

Conflict of interest statement

We declare that we have no conflict of interest.

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