Short Communication

Detection of Japanese Encephalitis Virus in Vector Mosquitoes in a Non-endemic Area, India

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Abstract

Japanese Encephalitis (JE) is a serious growing public health problem in India, gradually engulfing new areas. In north India, eastern districts of Uttar Pradesh had been highly endemic for JE since 1978. JE outbreak was reported to have jumped over 800 km distance from its earlier most endemic area (eastern parts of north India) to a location (Karnal) in the Haryana state of western parts of north India, during the year 1990, which never reported JE earlier.[1] Later, the disease gradually spread to its adjoining districts in the state. First time, from western Uttar Pradesh, 7 cases and 4 deaths due to suspected JE were reported in 2003 from Saharanpur district. In the subsequent year, 13 deaths due to suspected viral encephalitis were reported from 26th September to 23rd October, 2004 from one village namely Khekra, from Baghpat district of Uttar Pradesh which is about 128 km west of Saharanpur. A serosurvey carried out on 24th and 25th October, 2004 among the family members of dead persons revealed hemagglutination inhibition (HI) antibodies against JE and West Nile (WN) virus. Further, an outbreak of suspected JE was reported from Saharanpur district in 2005, with 212 cases and 157 deaths. This caused matter of great concern to extend further studies in Baghpat where prevalence of flavivirus infection was already recorded in the locality. For monitoring of arbovirus activities in an area, detection of virus in human sera is cumbersome, difficult and also not desirable. Therefore, as an alternative approach, detection of virus antigen in mosquitoes by antigen capture enzyme linked immunosorbent assay (ELISA) has provided a reliable tool to comprehend the types of virus circulating in nature.[2] Detecting arbovirus in mosquitos forms an important part of vector surveillance and may at times also serve as an early warning signal for outbreaks, if however linked with phonological and epidemiological studies. Vector infection and abundance were found to be good indicators of JE occurrence in surveillance studies conducted in South India.[3] An attempt was thus made to detect the presence of JE virus (JEV) antigen in vector mosquitoes by ELISA method from the encephalitis-affected area of Baghpat district and to provide evidence of circulating of Japanese Encephalitis virus (JEV) in natural animal-mosquito cycle.

Keywords: Japanese Encephalitis, *Culex tritaeniorhynchyus*, Baghpat, Uttar Pradesh, JE virus.

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Introduction

Baghpat district is in Uttar Pradesh state (28.57°N and 77.13°E). It has a population of 1164 thousand as per India census 2001. It is very closely located to (around 40 km) the national capital New Delhi towards north-west. The main occupation of the people is agriculture. The main crops grown here are wheat, sugarcane, maize, paddy, pulses and oilseeds. Two villages namely, Khekra (Prempuri) and Mubarikpur were undertaken for entomological studies.

Adult mosquitoes were collected from different outdoor locations from April to December, 2007 by Hop cage method[4] during day time. The outdoor resting locationswere mainly crop vegetation like rice, wheat, millet, sugarcane, mango orchards and fodder grasses. Larvae were collected from irrigation channels, ponds, paddy fields, ground water pools and ditches and were reared to adults in the laboratory for species identification. After identification, 22 pools of mosquitoes were made on the basis of sex-wise, date-wise and village-wise and these pools were stored at room temperature.

All mosquitoes were identified and species-, locality-, date- and sex-wise pools were made for the detection of JE virus by antigen-capture ELISA using monoclonal antibody (MAb), 6B4A-10 as capture antibody and MAb–peroxidase conjugate MAb 6B6C-1 as detector antibody.[5] Positive control (Source: NIV, Pune) and the homogenate of an uninfected adult mosquito pool of *Cx. tritaeniorhynchus* (laboratory-reared colony) as a negative control were used. Mosquito pools were considered positive for JE virus antigen if their optical density (OD) was greater than or equal to mean+4 standard deviation (SD) of the OD of the normal laboratory mosquito pools. The virus infection rate in mosquitoes was expressed as minimum infection rate (MIR) per 1000 females tested.[6] Haemagglutination inhibition (HI) has been employed as method for detection of viral antibody.[7]

In October, 2004, serological investigation of an outbreak of suspected viral encephalitis in Khekra, Bagpat district, showed moderate to high HI antibodies against JE and West Nile virus (Table 1) in seven asymptomatic contacts and one in suspected case, indicating prevalence of flavivirus infection in the locality.

Table 1.Results of Blood Samples of Suspects and Contacts Collected from District Baghpat, Uttar Pradesh in 2004 during an Investigation of Episode of Acute Febrile Illness

S. No.	Cases/Contacts	HI titers		Interpretation of Results
		JE	*WN*	
1.	Suspected case	*1:160*	*1:160*	Moderate level of HI antibodies against JE and WN virus
2.	Suspected case	*1:20*	*1:20*	Low level of HI antibodies against JE and WN virus
3.	Asymptomatic case	*<1:10*	*<1:10*	No HI antibodies against JE and WN virus
4.	Suspected case	*1:40*	*1:20*	Low level of HI antibodies against JE and WN virus
5.	Suspected case	*<1:10*	*<1:10*	No HI antibodies against JE and WN virus
6.	Suspected case	*<1:10*	*<1:10*	No HI antibodies against JE and WN virus
7.	Asymptomatic contact	*>1:1280*	*1:640*	High level of HI antibodies against JE and WN virus
8.	Asymptomatic contact	*>1:1280*	*>1:1280*	High level of HI antibodies against JE and WN virus
9	Asymptomatic contact	*1:160*	*1:160*	Moderate to low level of HI antibodies against JE and WN
10.	Asymptomatic contact	*1:160*	*1:160*	Moderate to low level of HI antibodies against JE and WN
11.	Asymptomatic contact	*1:160*	*1:160*	Moderate to low level of HI antibodies against JE and WN
12.	Suspected contact	*1:80*	*1:180*	Moderate to low level of HI antibodies against JE and WN
13.	Suspected contact	*1:180*	*1:140*	Moderate to low level of HI antibodies against JE and WN
14.	Asymptomatic contact	*1:10*	*1:10*	Low level of HI antibodies against JE and WN

Note: All samples were –ve for IGM antibodies to Dengue virus. Antibodies in contacts indicates prevalence of flavivirus infection in the locality

JE=Japanese Encephalitis

WN=West Niles

Further, the entomological surveys at Baghpat revealed the presence of two vector species of JE during the study period. Out of the total of 442 mosquitoes, 347 were of *Culex tritaeniorhynchus* and 95 of *Culex gelidus*. Out of the total of 347 adult *Cx. tritaeniorhynchus* mosquitoes, 79 were wild adult mosquitoes caught from outdoor resting places and 268 mosquitoes were reared from immature collected from different breeding sources of the same area.

However, out of the total of 95 *Cx. gelidus* mosquitoes, only one was adult caught from outdoor resting sites;

rest were from reared from larvae collected from different breeding sites from the field.

Out of the total of 347 of *Cx. tritaeniorhynchus,* 19 pools were made for JE virus detection. Of 19 pools of *Cx, tritaeniorhynchus*, 6 pools (3 male and 3 female pools) were tested from Mubarikpur and 13 pools (8 male and 5 female pools) from Khekra (Prempuri). However, only 3 pools of *Cx. gelidus* could be made*,* one each female pool was from Prempuri and Mubarikpur and one pool of male mosquito from Mubarikpur. Details of the test results are given in Table 2. Of the 13 pools of *Cx. tritaeniorhynchus* tested from Prempuri, one pool (7.6%) comprised outdoor resting wild caught mosquitoes was found positive for JE virus antigen collected from millet field in the month of August. Of the 6 pools from Mubarikpur, 2 pools (33%) were found positive for JE virus antigens in which one pool was with 2 male and other pools with 4 female mosquitoes. Mosquitoes of both positive pools were adult caught from outdoor resting sites in fodder grasses in the month of May. Three pools of (46 male and 49 female) *Cx. gelidus* were also tested; two from Mubarikpur and one from Prempuri, did not reveal JE virus antigen in them.

In most JE endemic regions in India, *Cx. tritaeniorhynchus* Giles is the principal vector of JE transmission and incriminated as vector of JE virus.[3,8-12]. First time in Baghpat, JE virus was detected in *Cx. tritaeniorhynchus*. In total, 15.8% harbored JE virus. Similarly in Delhi, 16.66% harbored JE virus[10] where first time JE confirmed indigenous cases were reported in 2011. However, in JE endemic Cuddalore district from South India,[13] only 2.1% pools of *Culex vishnui* subgroup mosquitoes were found positive for JEV antigen.

Of the total three positive pools of *Cx tritaeniorhynchus* from Baghpat, two pools (66.66%) comprised of wild adult caught males, suggesting vertical transmission of JE virus in the vector mosquitos in the non-endemic areas. Vertical transmission in *Cx. tritaeniorhynchus* was also reported from Delhi.[10] As vertical transmission was earlier reported,[14-17] the mosquitoes inherited the infection by vertical transmission from the infected parents. Vertical transmission may be an additional mechanism for the maintenance of JE virus in nature so it shows persistence of JE virus in vector mosquitoes. The occurrence of vertical transmission of JE virus in vector mosquito species might have epidemiological significance.[14]

In the present study, MIR of *Cx. tritaeniorhynchus* in non-endemic district Baghpat was found to be much higher (8.6) than that 6.06[4] as reported from nearby JE endemic, Karnal district of Haryana. The average MIR of 1.4 with a peak MIR of 5.6 was reported also from JE endemic villages from Cuddalore district of Tamil Nadu, South India.[18] As JE is endemic in both Cuddalore and Karnal districts where was the major vector.[4,18] Similar to our report, high MIR of 1.3 was reported from a non-endemic zone in Tamil Nadu, South India, as compared to nearby endemic zones,[13] indicating an increase of the circulation of JEV in non-endemic area but no human case was reported in that area. The ratio of pig to cattle was 1:400 in Thanjavur whereas in the neighboring JE endemic areas it was 1:4.[19] Further, they explained that since the vector species of JE are zoophilic, JEV does not multiply in cattle, hence JEV did not spill over to humans.[20] It was also observed during the present study that amplifying host-pig population was found very less at close proximity to human in study localities of Baghpat. JE virus is maintained in nature by a basic culex-bird cycle with amplification in a culex-pig cycle.[21] In order to develop a predictive model for JEV infection to humans, the possible temporal relationship of porcine infection to human encephalitis cases was studied[22,23] and there was correlation between the degree of porcine seroconversion and human cases as in Kolar district, Karnataka.[24]

In northern India, JE has been repeatedly reported from eastern part of Uttar Pradesh since 1978, but the disease spread to Haryana in 1990[1] and then it has further spread to other western parts of Uttar Pradesh, Saharanpur, since 2003 and Muzaffarpur since 2005.[25] First time, JE was also reported in 2004 from Rohtak, Haryana, which is the neighboring district of Baghpat. The eastern Yamuna canal irrigates Saharanpur, Muzaffarpur, Rohtak and Baghpat. The irrigated land in the areas becomes marshy and water logged to become receptive for JE. Japanese encephalitis cases have already been reported from these districts except from Baghpat. However, in October, 2004, serological investigation of an outbreak of suspected viral encephalitis in Baghpat district showed high level of HI antibodies against JE and West Nile virus, indicating prevalence of flavivirus infection in the locality. Present result indicates that in Baghpat district there is prevalence of JE vectors with positive JEV antigens with high MIR as compared to other endemic areas but no confirmed human JE case is reported, although prevalence of flavivirus infection recorded in the locality which confirmed that the occurrence of silent JE transmission in the non-endemic area, it may be due to sub lethal doses of JEV.[13] The disease is known to be spreading in newer areas. The spread of JE infection in newer areas is marked by extensive epidemics; however, the pre-existence of the JE virus in affected

areas is not curtained, there might, however, to be less grade of transmission before outbreak of epidemics. This is a warning signal for JE outbreak in Baghpat, if ecological conditions are favorable for proliferation of infected vector mosquitoes, and further, for availability of amplifying hosts (pigs) for transmission of JE in the areas. Therefore, a strong surveillance is recommended in these areas.

Table 2.JE Virus Detected in Mosquitoes Collected from Baghpat District, Uttar Pradesh

S. No.	Locality	Mosquito Species	No. of Pools Tested	Mosquitoes Tested			No. of Pools Positive
				Male	Female	Total	
1	Khekra (Prempuri)	*Cx.tritaeniorhynchus*	13	129	178	307	1*
		Cx. gelidus	1	0	48	48	0
2	Mabarikpur	*Cx.tritaeniorhynchus*	6	28	12	40	2**
		Cx. gelidus	2	46	1	47	0

*20 male mosquitoes; **1 pool with 2 male and other pools with 4 female mosquitoes

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Conflict of Interest: None

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