Following first recorded JE endemic in India during 1973 reported in West Bengal and its subsequent recurrence in 1976, 1978 and onwards mosquitoes were collected from Endemic zone. Although JEV isolated from 10 species of mosquitoes in India belonging to the genus *Culex, Anopheles* and *Mansonia*. It was established that principal vectors are the mosquitoes belonging to the different species of Culex. Repeated isolations of JEV from *Cx. vishnui* in successive epidemics of 1973, 1976, 1978, 1979 in West Bengal suggest the possibility of this mosquito being the vector of JE virus in West Bengal.

A study was undertaken to study the biology of *Cx. vishnui* in JE affected rural West Bengal, and the role of other related species has also been investigated and in comparative study with a non endemic area adopting the same standard parameters with a view to know and gauge the causation of JE in an endemic region and also to make an attempt to know multifaceted factors responsible for its perpetuation in one area (Endemic) and abstinence in the other area (Non-endemic).

The study was roughly designed to detect the component of *Cx. vishnui* complex i.e. *Cx. vishnui, Cx.pseudovishnui* and *Cx.tritaeniorhynchus* in both endemic and non-endemic regions. The distribution, resting preference in different biotopes, seasonal prevalence of adults as well as immatures, blood meal analysis and relation with humidity temperature and rainfall.

Infected mosquitoes actually transmit the virus to hosts in nature in endemic area. The activity of the virus throughout the year in an endemic and non-endemic area has been evaluated by sentinel study.

In the present study JE in two areas in West Bengal (endemic and Non-endemic) has been monitored throughout the year by examining the sera of sentinel birds for the detection of JE antibody. At the same time an attempt has been made to correlate these findings with the prevalence of *Cx. vishnui* complex mosquitoes caught per man hour from Human dwellings and Cattle shed and prevalence of Japanese encephalitis (from official record). A reliable method to know whether viral activity is present or not in an area
has been developed. This method is based on positivity of sentinel chicks in different season through months and it has been shown that sentinel chicks can predict the activity of virus.

The salient observations made by the study are being summarised below.

(i) The study was conducted in an endemic village Nudipur of Memari block in Burdwan district about 90 km from Calcutta and a non endemic village Sitarampur of Magrahat block, South 24-Parganas district about 28 km from Calcutta.

The Central Laboratory was situated in the Calcutta School of Tropical Medicine. Vector identification was done in the Department of Medical Entomology while serological work was performed in the Department of Virology.

The study was conducted in all the three seasons, the rainy seasons, (July - October), the Winter season (November to February) and the Summer season (March - June).

Adult mosquitoes were collected fortnightly 6 am to 7 am in three fixed cattle sheds and three fixed human dwellings from both villages by applying hand collection method of W.H.O. The collections were taken to the central laboratory at School of Tropical Medicine, Calcutta for identification and blood meal analysis.

(2) Immatures of mosquitoes were collected fortnightly between 6 am to 7 am in the paddy field, ponds and fallow fields by dipping method of W.H.O. The collections were taken to the central laboratory for identification by observing the combplate and pectin.

(3) Non immune sentinel chicks (2-8 days) from non endemic zone (Calcutta) were transported to the study area (endemic zone, Nudipur and non-endemic zone Sitarampur) and exposed to the vector mosquito bite for 15 days. Chicks were placed in the central part as also the four corners of the village. Chicks were then taken to the Central Laboratory to detect the appearance of JE antibody in their sera by using Haemagglutination Inhibition test (HAI). Positivity of JE antibody in chick blood during the period June 1994 to May 1995 was correlated with apperence of JE cases within the human population of that area. The diagnostic titre was taken as 1 : 20.

(a) A total 1440 adult mosquitoes of *Cx.vishnui* complex were collected from Nudipur (880) endemic zone in Memari and from Sitarampur (560) a non-endemic zone. The collection is more in Nudipur endemic zone (PMHD 18.2) than in Non-endemic zone Sitarampur (PMHD 11.6) and the ratio is 1.57 : 1.

Number of collection of *Cx. vishnui* (438, PMHD 9.1) and *Cx. tritaeniorhynchus* (342, PMHD 7.1) is more in endemic zone than *Cx. vishnui* (188 PMHD 3.9) and *Cx.*
tritaeniorhynchus (246 PMHD 5.1) of non-endemic zone, where as in case of Cx. pseudovishnui collections is more in Non-endemic zone (126 PMHD 2.6) than that of endemic zone (100, PMHD 11.4).

(2) The three species of Culex vishnui complex i.e. Cx. vishnui, Cx. pseudovishnui and Cx. tritaeniorhynchus prefered to rest in cattle shed than in human dwellings both in endemic zone (71.82%) and non-endemic zone (60.4%).

(3) In endemic zone collection of Cx. vishnui were highest (49.8%) followed by Cx. tritaeniorhynchus (33.8%) and Cx. pseudovishnui (11.4%) where as in Non-endemic zone Cx. tritaeniorhynchus occupied the first position. Subsequent position were taken by Cx vishnui (33.5%) and Cx. pseudovishnui (22.5%).

(4) The maximum collection of Cx. vishnui was found in summer both in endemic (PMHD 11) and non-endemic (PMHD 5.1), Cx. pseudovishnui showed maximum prevalence in summer both in endemic (PMHD 4) and non-endemic zone (PMHD 4.5). Where as Cx. tritaeniorhynchus showed highest prevalence in Rainy (PMHD 10.4) in endemic zone and in Summer in Non-endemic zone (PMHD 8.25).

Lowest collection of Cx.vishnui was found in the winter (PMHD 6.4) in endemic and the rainy (1.75) in non endemic zone (PMHD 1.75) which minimum collection of Cx.pseudovishnui was noted in the rainy in endemic (PMHD .75) and Non endemic (PMHD 1.1) zone.

Minimum collection of Cx. tritaeniorhynchus was observed in winter & summer in endemic (5.5) and in rainy in Non-endemic (2.25) region.

So it is seen that from summer to the rainy Cx.vishnui showed marginal decrease but Cx.tritaeniorhynchus showed enormous increase in Endemic zone. On the other hand in non-endemic zone both Cx.vishnui and Cx.tritaeniorhynchus showed sharp drop in the rainy season.

(5) Immature distribution showed a higher collection (2812) in endemic region when compared to non-endemic zone (1325) and the lower value was due to (a) lack of Cx.vishnui species (39 in non-endemic compared to 247 in endemic) (b) lack of Cx.tritaeniorhynchus (833 in non-endemic compared to 2201 in endemic zone) although value for Cx. pseudovishnui was formed slightly higher (4.53 against 364) in Non-endemic zone).

But the fact that this was not reflected in availability of corresponding proportion, of adults to that extent.
In non-endemic zone on seasonwise analysis for both *Cx.vishnui* and *Cx.tritaeniorhynchus* values were comparable in non-endemic zone 17 (rainy) 11 (winter) 11 (summer) for *Cx. vishnui* species and 350 (Rainy) 278 (winter 205(summer) for *Cx.tritaeniorhynchus*. But in endemic zone there were wide variations from season to season. For *Cx.vishnui* species the pattern from the summer to the rainy to the winter season could be described as low (29) to high (153) to moderate (65). But for *Cx.tritaeniorhynchus* this pattern was better described as high (1027) to relatively low (575) then maintained (599). The essential feature was maintenance of the value in the rainy season (575) in case of *Cx.tritaeniorhynchus* into the winter season and later on very high values (1027) preceding rainy season.

(6) In endemic zone greater number of immatures of *Cx.vishnui* (46.6%) *Cx.pseudovishnui* (42.3%) and *Cx.tritaeniorhynchus* (54.7%) obtained in paddy fields compared to ponds and ditches of endemic zone. Similarly, in non endemic zone immatures of *Cx.vishnui* (48.7%), *Cx.pseudovishnui* (58.5%) and *Cx.tritaeniorhynchus* (59.3%) were found in greater number in fields than ponds and ditches.

The immature collection in endemic zone (2812) were more than non-endemic zone (1325) and the increased value was due to a remarkably higher value of *Cx.vishnui* species (247 compared to 39) and *Cx.tritaeniorhynchus* (2201 compared to 833).

Strangely, the value for *Cx.pseudovishnui* was higher in Non-endemic (453) than in endemic zone (364).

*Cx.tritaeniorhynchus* is seen to have high values both in endemic (78.3%) and non endemic zone (62.9%). But the fact that this was not reflected in availability of corresponding population of adults to that extent suggests different chances of immature survival for this species.

Seasonwise analysis showed that *Cx.vishnui* species and *Cx.tritaeniorhynchus* spend in non-endemic zone have almost nearest value in Rainy, Winter and Summer season i.e. in case of *Cx.vishnui* (17, 11, 11) and *Cx. tritaeniorhynchus* (350, 278, 205). But in endemic zone there were wide variations from season to season.

For *Cx.vishnui* species the pattern from summer to the rainy to the winter season would be described as low (29) to high (153) to moderate (65). But for *Cx. tritaeniorhynchus* pattern was better described as high (1027) to relatively low (575) then maintained (599). The essential feature was maintenance of the value in the rainy season (575) in case of *Cx.tritaeniorhynchus* into winter season (591) and later on very high value (1027).
preceeding rainy season.

(7) It has been observed that in endemic zone, greater number of Cx.vishnui (46.6%), Cx.pseudovishnui (42.3%) and Cx.tritaeniorhynchus (54.7%) obtained in the paddy fields compared to ponds (42.5%, 32.7%, 22.7%) and ditches (10.9%, 25%, 22.6%).

The picture in Non-endemic zone showed greater number of Cx.vishnui, Cx.pseudovishnui and Cx.tritaeniorhynchus (48.7%, 58.5%, 59.3%) obtained in the paddy fields compared to ponds (33.3%, 20.5%, 20.5%) and ditches (18%, 21%, 20.2%) respectively.

Out of 3 species Cx.tritaeniorhynchus showed higher prevalence in field (i.e. 54.7% against 46.6% (Cx.vishnui), 42.3% (Cx.pseudovishnui) in endemic, and (57.3% against 48.7% (Cx.vishnui), 20.5% (Cx.pseudovishnui) in non-endemic region.

Host preference of Culex vishnui complex.

Results of blood meal analysis showed that 85% of wild caught Cx.vishnui complex in endemic zone were tested positive in endemic zone both in human dwelling and cattle shed. In non endemic zone 86% were tested positive in human dwellings and 87% were tested positive in cattle shed.

It was observed that throughout the year Bovine positivity (63.5%) topped the list followed by Human positivity (21.5%), Porcine positivity (10.4%) and avian positivity (4.6%).

In endemic zone maximum positivity was encountered in summer (42.8%) followed by winter (41.1%), rainy (36.1%) whereas in non-endemic zone maximum positivity was noticed in the winter (43.6%) followed by winter (41.5%), rainy (14.9%). Difference in positivity is negligible in the summer and the winter both in endemic and non-endemic zone.

Monthwise positivity of JE antibody in sentinel chicks

<table>
<thead>
<tr>
<th>Months</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>Aug</th>
<th>Sept</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endemic area chick positivity (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non Endemic area chick positivity (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
It is seen from the table that there were two months April, May during which antibodies against JE were detected in sentinel chick in the endemic zone.

The overall chick positivity was 3.85% Taking the data separately for three seasons

a) In the rainy season (July to October) the positivity was 18.75
b) In the summer season (March to June) the positivity was 4%
c) In the winter season (November to February) the positivity was 0%

The two periods of two months each in which sentinel chick positivity was noted occurred twice.

(i) Once in the rainy season (September - October)
(ii) Once in the summer season (April - May)
(iii) But no such period occurred in the winter season.

It was interesting to note that in a previous study in this area, the overall (annual) sentinel chick positivity rate was 26.2%.

(i) In the rainy season 22.5%
(ii) In the winter season 24%
(iii) In the summer season 29.5%

The observation were established in the previous study. But the present study shows the maximum seroconversion of chicks occur during the rainy seasons (18.75%). This indicates the shifting to maximum seroconversion indicating the shifting of the time of breakout of the disease.

Absence of seroconversion in sentinel chick in winter appears to be a deviation from the previous picture. Thus this type of study predicts whether this disease is perineal in the area or incoming.

During the time of Boropaddy cultivation, the vector density is increased in March and April in this connection the virus may transmit from wild bird to perineal reservoir and to the domestic chicks but due to short duration of maintenance of vector density would not be able to spill over infection in human.

In endemic zone antibody in sentinel chick was well detected in April - May and September - October. But it cannot be ruled out presence of antibody in the months June to August. The latter observation pointing to perineal transmission during the said period as studied by previous investigation (Bhattacharyya et al. 1990).

1. Sitarampur, Magrahat South 24-Parganas is not a mass paddy cultivated area
The vector is there but it does not reach the optimum density to transmit the infection in the domestic chicks and human although arised birds are there with infection.

2. In the non endemic zone, in the absence of natural transmission, there was no seroconversion in sentinel chicks at any time during the year.

Monthwise density (PMHD) of different species of *Cx.vishnui* complex in endemic zone.

<table>
<thead>
<tr>
<th>Months</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>Aug</th>
<th>Sept</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cx.vishnui</em></td>
<td>7.25</td>
<td>8</td>
<td>25</td>
<td>17</td>
<td>0</td>
<td>2</td>
<td>8</td>
<td>13</td>
<td>12</td>
<td>7</td>
<td>5</td>
<td>4.75</td>
</tr>
<tr>
<td><em>Cx.pseudovishnui</em></td>
<td>0</td>
<td>0</td>
<td>11.5</td>
<td>4.5</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td><em>Cx tritaeniorhynchus</em></td>
<td>9.5</td>
<td>0</td>
<td>10.25</td>
<td>10.75</td>
<td>0</td>
<td>1</td>
<td>11.25</td>
<td>12.25</td>
<td>9</td>
<td>9</td>
<td>6.25</td>
<td>6.25</td>
</tr>
</tbody>
</table>

PMHD equal and exceeding critical value '8' to be taken as Peak.

*Cx. vishnui* sp. showed two peaks. One in February, March, April and the other in July, August & September.

During these 4 months, values are equal or exceeding the critical value of 8.

The peak in February, March, April was higher but was followed by an abrupt fall to zero in May. The peak in July, August, September was not so high but the subsequent fall was of lesser magnitude. *Cx. pseudovishnui* had low values almost throughout the year - with values of zero in six out of 12 months, and values not exceeding 2 in three other months. Relatively high values (11 in March followed by early fall to 4.5 in April) were seen only once in the year - not twice as *Cx. vishnui* species. In the case of *Cx tritaeniorhynchus* the monthwise pattern had more similarities with that of *Cx. vishnui* species but the difference was also noteworthy.

As in *Cx. vishnui* species there were two peaks. One peak was in March - April (Same as *Cx. vishnui* sp.) and the other peak was in July - August (one month earlier than *Cx. vishnui* sp.).

As in *Cx. vishnui* species there was an abrupt fall to zero in May - The second peak in July - August was somewhat higher than in March - April but the fall was much slower with values in excess of 8 persisting for another two months. On the whole for *Cx
tritaeniorhynchus values exceeded 8 for seven months in the year, including one month during the winter season.

Relation between critical PMHD of vector and occurrences of human cases

<table>
<thead>
<tr>
<th>Months</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>Aug</th>
<th>Sept</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human case in the Block concerned</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Human cases in the District As a whole</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>6</td>
<td>29</td>
<td>30</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Density of Cx.vishnui</td>
<td>7.25</td>
<td>8.25</td>
<td>25</td>
<td>17</td>
<td>0</td>
<td>2</td>
<td>8</td>
<td>13</td>
<td>12</td>
<td>7</td>
<td>5</td>
<td>4.75</td>
</tr>
<tr>
<td>Density of Cx.tritaeniorhynchus</td>
<td>9.5</td>
<td>0</td>
<td>10.25</td>
<td>10.75</td>
<td>0</td>
<td>1</td>
<td>11.25</td>
<td>12.25</td>
<td>9</td>
<td>9</td>
<td>6.25</td>
<td>6.25</td>
</tr>
</tbody>
</table>

Analysis of the timing of high vector (Cx. vishnui and Cx.tritaeniorhynchus) density and occurrence of human JE cases also show a distinct patterns.

(1) First peak :
   a) High Cx. vishnui density occurred in February, March and April.
   b) High Cx. tritaeniorhynchus density occurred in January, March, April.
   c) No human cases were seen.

The explanation could be rapidly falling vector density making spillover of infection to human impossible to transmit, infact the vector fall to "0" in May.

(2) Second peak :
   a) High vector density of Cx. vishnui was noted in July, August & September.
   b) High values for Cx. tritaeniorhynchus was noted from July to October.

(3) Human cases were reported in August i.e one month after rise of Cx. vishnui.
& Cx. tritaeniorhynchus density.

(4) Tracing this observation in the subsequent months beyond September - density of Cx. vishnui species fell to 7 while density of Cx. tritaeniorhynchus remained above 8 and Human cases continued to occur.

(5) Density of Cx. tritaeniorhynchus fell below the critical value beyond October and the last case in the study area occurred in November. Cases of JE these were reported till one month after fall of density of Cx. tritaeniorhynchus.

(6) So high vector density requires to be sustained for occurrence of JE cases.

Sentinel chicks can predict the activity of virus

We have developed a reliable method to know whether the viral activity is present or not in an area. This method is based on positivity of sentinel chicks in different seasons through months. This method is cheap, easier and can be effectively utilised in predicting the outbreak of the diseases in human.

It has been observed throughout the study period that the vector species responsible for transmission of Japanese encephalitis was found in abundance in Sitarampur (Non endemic zone). But antibodies to Japanese encephalities was not noted in sentinel chicks in any month side by side no case of Japanese encephalitis was found in the area. Hati (1986) observed the incidence of infection in sentinel chicks exposed at monthly intervals the prevalence of antibody in chick and the occurrence of clinical infection in human population (August 1981 to August 1982).

Thus a correlation between sentinel chick positivity and JE cases established in an area of persistent vector abundance (Cx. vishnui complex). In other words positivity in sentinel chick (Antibody to JEV) can predict the onset of the disease process (JE) and as there is no recorded case of sentinel chick positivity in Sitarampur so it may be called a non endemic zone.

Thus sentinel chick denotes the appearance of JE antibody in nature. In this respect the role of vector is also correlated. Fluctuation of density of vector (PMHD) is very much important as it has been observed increased value of vector density is closely related
Hati (1990) stated, that it was assumed that with minimum per man hour density of 7.58 of *Cx. vishnui* in human habitations JE epidemic may start in favourite seasons. The future out break may further be correlated with per man hour density of vector in human habitation.

It is seen in the present study that antibody in sentinel chick appeared at a high level in low vector density context (composition) resulting in non occurrence of JE cases, side by side low level of antibody in sentinel chick (i.e. perennial transmission of the virus in its maintenance cycle) in a high vector density population may lead to appearance of human infection.

Present data showing antibody in sentinel chick well detected in April - May and September - October do not conclude absence of antibody in the months June to August. The latter observation pointing to perennial infection transmission during the said period as studied by previous investigators. Bhattacharya S *et al* (1990) stated perennial transmission of JE virus in sentinel going on which conclusion is reinforced by the consistant prevalence of antibodies in wild birds.

Thus two factors mainly sentinel chick positivity and high vector density over a prolonged period is essential for appearance of human infection of JE.

"Therefore, only when the mosquito population has grown substantially and a high level of virus is present in it, the virus will spill over to human population giving rise to epidemic outbreak (Hati AK 1990).

According to this analogy *Culex vishnui* is stated to be the main vector of Japanese encephalitis yet the role of *Cx. tritaeniorhynchus* should be accounted as equally important.