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EXPERIMENTAL STUDIES ON THE SUSCEPTIBILITY OF *AEDES VITTATUS* TO DENGUE VIRUSES

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Summary. - *Ae. vittatus* mosquitoes were infected by oral route and by intrathoracic inoculation with dengue (DEN) viruses and tested for the presence of dengue virus antigen in their head squashes and salivary glands by indirect immunofluorescence. The results indicate that this species was susceptible to all four types of DEN viruses and supported the growth of DEN-2 virus.

Key words: dengue viruses; Aedes vittatus; immunofluorescence

Aedes aegypti, a day biting mosquito is the principal vector of DEN viruses in most of tropical and subtropical countries, including India (Ramachandra Rao, 1964). *Ae. vittatus,* another common mosquito species, which is known to bite man, occurs in many parts of India and is closely associated with human habitats (Ramachandra Rao and Rajagopalan, 1957; Bhat, 1975). Its role as a vector for yellow fever virus in some parts of Africa was well documented (Satti and Haseeb, 1966) but its vector competence for other arboviruses in India has been limited to a study with Chikungunya virus (Mourya and Banerjee, 1987). This mosquito species was suspected as a vector for DEN virus (Ramachandra Rao, 1964), but its suspectibility to DEN virus has not been determined yet.

The *Ae. vittatus* mosquitoes employed in the present study were obtained from a laboratory colony maintained at National Institute of Virology, Pune. All the four sero-types of DEN viruses were used, the source of isolation and passage histories of the strains are given in Table 1. The mosquitoes were infected by both parenteral as well as by oral routes. For infecting the mosquitoes by parenteral route three to four days old female mosquitoes were inoculated introthoracically, as described by Rosen and Gubler (1974). After inoculation, they were held in plastic jars covered with mosquito netting. They were provided with cotton pledgets soaked in 10 % glucose solution and incubated at 28 °C±1 and relative humidity of 80-85 %. To infect the mosquitoes by oral route, freshly defibrinated chicken blood was collected and mixed with the virus. Three to four days old, overnight starved mosquitoes were fed on this blood-virus mixture through fresh chicken skin. Fully fed mosquitoes were

held in similar plastic jars as the inoculated ones. The post feeding blood-virus mixture was titrated in infant mice. All the 4 types of dengue viruses were used for infecting the mosquitoes by both the routes. The mosquitoes were tested for presence of DEN viral antigen at different post-infection (p. i.) intervals. Always 10 mosquitoes were tested on day 5 p. i. and on every alternate day thereafter, upto day 15 p. i. in case of inoculated ones and upto day 21 p. i. for orally infected mosquitoes. Viral antigen was detected by indirect immunofluorescence test (IIFT) performed on the head squashes as well as on the salivary glands of the mosquitoes using DEN-2 hyperimmune serum raised in rabbits. This immune serum was earlier shown to react with all the 4 types of dengue viral antigen. The immunoconjugate against rabbit serum was procured from Nordic Laboratories, Tilburg, Netherlands.

To follow the growth pattern only DEN-2 virus was used. Mosquitoes were infected by introthoracic inoculation as described above. Three mosquitoes were tested on day 0, day 1 and then on every alternate days until day 15 p. i.

Table 1. Dengue virus strains used for this study

DEN type	Virus strain designation	Source	Year of isolation	Locality	Passage level
DEN-1	Hawaiin	Human Ser.	1945	Hawaii	Not known
DEN-2	TR 1751	Human Ser.	1953	Trinidad	P-12
DEN-3	633798	Human Ser.	1963	Thailand	P-25
DEN-4	611319	Human Ser.	1961	Vellore	P-31

Table 2. Detection of dengue viral antigen (1 to 4) in Ae. vittatus infected by intrathoracic inoculation

Days p.i.	DEN-1		DEN-2		DEN-3		DEN-4	
	S.G.	H.S.	S.G.	H.S.	S.G.	H.S.	S.G.	H.S.
5th	1/10	1/10	1/10	1/10	1/10	0/10	0/10	0/10
7th	6/9	2/10	8/8	7/10	5/8	2/10	3/10	1/10
9th	7/10	4/10	9/9	8/10	4/8	3/10	6/10	4/10
11 th	9/9	9/10	9/10	9/10	6/8	9/10	9/10	9/10
13 th	9/10	9/10	10/10	10/10	8/10	7/10	8/10	8/10
15th	10/10	8/10	10/10	10/10	6/6	6/7	9/10	8/10

S.G. = Salivary gland, H.S. = Head squash

Suspension of each individual mosquito was prepared in 1 ml of 0.75 % bovine albumin phosphate saline (BAPS). Serial ten fold dilutions of these suspensions were made in BAPS and inoculated into groups of uninfected *Ae. aegypti* to determine the virus titre. The mosquitoes were held for 12 days in an insectary for incubation and examined for the presence of DEN-2 viral antigen by IIFT on their head squashes. The titre of the virus was calculated by the method of Reed and Muench (1938) and was expressed as 'dex' (Haldane, 1960).

Results of DEN viral antigen detection in head squashes and salivary glands in *Ae. vittatus* infected by introthoracic inoculation are shown in Table 2. On day 5 p. i. the DEN-1, 2 viral antigens were detected in both the head squash and the salivary glands of a single mosquito, whereas DEN-3 viral antigen was detected in the salivary gland only. No mosquito was positive for DEN-4. From day 7 p. i. onwards infection was detected with all the 4 types of dengue viruses in both the head squashes as well as the salivary glands. There appeared to be a gradual increase in the rate of infection with the passage of time. Near 100 % infection was observed from day 11 till the end of the experiment. It was also observed that the rate of infection in the salivary gland was consistantly higher than in the head squashes with all the 4 types of dengue viruses.

Table 3. Detection of dengue viral antigen (1 to 4) in Ae. vittatus infected by oral feeding

Days p.i.	DEN-1		DEN-2		DEN-3		DEN-4	
	S.G.	H.S.	S.G.	H.S.	S.G.	H.S.	S.G.	H.S.
5th	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
7th	0/10	0/10	0/8*	1/10	0/10	0/10	0/10	0/10
9th	0/10	0/10	0/10	0/10	0/7*	2/10	0/10	0/10
11 th	2/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
13 th	1/10	1/10	1/10	0/10	0/10	0/10	0/10	0/10
15th	0/9	0/10	4/9	4/10	0/9	0/10	2/9	2/10
17th	1/7*	2/10	0/5	0/5	0/5	0/5	0/5	0/5
19th	1/5	1/5	1/5	1/5	0/5	0/5	0/5	0/5
21th	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Post feeding titre**	5.1 dex		5.8 dex		6.4 dex		6.8 dex	

S.G. — Salivary gland, H.S. — Head squash
* S.G. of respective +ve H.S. lost during processing.
** Titre expressed as dex LD_{50} in infant mice.

Table 4. Growth of DEN-2 virus in Aedes vittatus by intrathoracic inoculation

Day post infection	Virus titre* in individual mosquitoes	Average titre*
0	0.0	0.0
3	0.0	0.0
5	2.2, 2.4, 2.5	2.3
7	0.0, 2.2, 2.4	1.5
9	2.3, 2.5, 2.6	2.4
11	1.5, 1.7, 2.1	1.8
13	2.0, 2.1, 2.6	2.2
15	1.6, 1.6, 2.4	1.8

*Titre expressed as dex ID_{50} in mosquitoes.

The results of the detection of dengue viral antigen in head squash and salivary glands of *Ae. vittatus* infected by oral route with all four types of DEN viruses are given in Table 3. The earliest infection was detected on day 7 p. i. at a low rate. There was no uniformity of infection with any of the viruses but in the positive mosquitoes, both head squash and salivary gland were positive.

The titre of DEN-2 virus after introthoracic inoculation of 3 mosquitoes tested on different days p. i. (Table 4). On the day of inoculation, i. e. day 0 and day 3 p. i. no virus growth was observed. Though some of the mosquitoes on day 0 showed traces of residual virus, its titre could not be calculated. Virus growth was observed on day 5 p. i. when the average titre was 2.3 dex. Maximum growth was observed on day 9 p. i., with a titre of 2.4 dex., then the titre ranged between 1.8 dex to 2.2 dex till day 15 p. i.

We found that *Ae. vittatus* is susceptible to all four types of dengue viruses. The rate of infection was markedly lower in orally infected mosquitoes (<5 %) as compared to inoculated ones (>63 %). The difference in the rate of infection may be due to the'gut barrier', but the exact reason is still not clearly understood. The detection of infection in salivary gland is an indication of the vector competence of the mosquito species (Chamberlain, 1968), thereby implying that *Ae. vittatus* is capable of transmitting the virus to a susceptible host by bite. It comes from the study of growth pattern of DEN-2 virus in individual mosquitoes, that this mosquito species was competent in supporting DEN-2 virus replication after infection by introthoracic route. Though the vector competence of *Ae. vittatus* under experimental conditions has been found to be low, it can act as vector of dengue viruses. Even with *Ae. aegypti,* which is the known vector of dengue viruses, it has been shown that the rate of infection in mosquitoes through artificial membrane feeding is much lower as compared with parenteral inoculation. It has also been shown in *Ae. aegypti* that the rate of

infection is slightly higher when the mosquitoes are fed on a viraemic host (human), even though the titre of the circulating virus in the blood may not be as high as artificial virus-blood feeding mixture. We conclude that even though the rate of infection of the orally infected mosquitoes was low, the possibility of their acting as a natural vector cannot be ruled out.

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