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SUSCEPTIBILITY OF FOUR SPECIES OF MOSQUITOES TO CHANDIPURA VIRUS AND ITS DETECTION BY IMMUNOFLUORESCENCE

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Summary. - Susceptibility of *Culex tritaeniorhynchus, Cx. Bitaeniorhynchus, Cx. quinquefasciatus,* and *Aedes aegypti* to Chandipura (CHP) virus was compared after parental inoculation of the mosquitoes. Virus detection was done by indirect immunofluorescence (IF). CHP antigen in head squashes of all the four species was seen at 24 hr post infection (p. i.). The mosquitoes supported the virus growth and transmission by bite to 2 days old suckling Swiss albino mice. *Ae. aegypti* which was found the most susceptible mosquito species for CHP virus can be used as a substitute for laboratory mice.

Key words: Chandipura virus; mosquito susceptibility; vector competence; immunofluorescence

Introduction

Chandipura (CHP) virus (family Rhabdoviridae, genus *Vesculovirus*) was originally isolated from the serum of patients during an outbreak of febrile illness in Nagpur city, Maharashtra, India, in 1965 (Bhatt and Rodrigues, 1967; Rodrigues *et al.*, 1972). Two other viruses, *viz.* dengue type 4 and chikungunya were also isolated during this epidemic. *Aedes aegypti* mosquitoes processed during the epidemic yielded dengue and chikungunya viruses but no CHP virus was isolated. Subsequently, CHP virus was isolated from phlebotomine sandflies collected at Aurangabad, Maharashtra, India (Dhanda *et al.*, 1970).

Recently mosquito inoculation and immunofluorescence were successfully used for detecton and isolation of dengue, Japanese encephalitis and chikungunya viruses (Kuberski and Rosen, 1977; Ilkal *et al.*, 1984; Mourya and Banerjee, 1987; Mourya *et al.*, 1989). It is a simple, rapid and highly sensitive technique. The present communication reports the multiplication of CHP virus in four species of mosquitoes infected by parenteral inoculation and its detection by immunofluorescence. Information is also provided on the vector competence of these mosquitoes for CHP virus.

Materials and Methods

Mosquitoes. The species of mosquitoes used in the study were *Culex tritaeniorhynchus, Cx. bitaeniorhynchus, Cx. quinquefasciatus*, and *Ae. aegypti.* They were obtained from the colonies maintained in this laboratory.

Virus. CHP virus strain 653514, isolated from the serum specimen collected from a human case during the acute phase of illness in Nagpur in 1965 was used. It had undergone 3 intracerebral (i. c.) passages in Swiss albino mice. The virus pool had an i.c. titre of 7.5 dex LD_{50}/0.02 ml.

Mosquito susceptibility and vector competence. Mosquitoes were infected by parenteral inoculation with serial ten fold dilutions of the virus suspension starting from 10^{-1} to 10^{-8}, each mosquito receiving approximately 0.2 μl of inoculum. Five mosquitoes from the batch inoculated with 10^{-1} dilution were tested six hourly each from 12 up to 54 hr and then between day 3 post infection (p. i.) to day 7 p. i. Of the remaining batches 5 mosquitoes each were tested at 24 hr, 30 hr, and 36 hr p. i. All the inoculated mosquitoes were held on 10% glucose solution at 28 - 29 ^{0}C and 80 - 85% R. H.

The head squashes of inoculated mosquitoes were tested by indirect immunofluorescence (IF) technique using CHP hyperimmune serum raised in mice as described by Dhanda and Ilkal (1985). For studying the vector competence, the surviving mosquitoes from the above experiment were used. Mosquitoes inoculated with 10^{-1} dilution of virus suspension were allowed to feed overnight on suckling 2-day-old mice on day 7 p. i. The mice were subsequently observed daily and deaths were registered. The brains of sick mice were tested by "quick" complement fixation (QCF) test as described by Pavri and Shaikh (1966) in the second mouse passage.

Virus growth. Another experiment was carried out to follow the growth of the virus in all four species of mosquitoes. The dose of the virus was determined on the basis of the previous experiment. *Cx. bitaeniorhynchus* and *Cx. quinquefasciatus* received 3.5 dex, whereas *Cx. tritaeniorhynchus* and *Ae. aegypti* received 2.5 dex LD_{50}. Five mosquitoes were tested on alternate days starting from day 1 p. i. Suspensions of individual mosquitoes were prepared in one ml of 0.75 % bovine albumin phophate saline (BAPS). Ten fold serial dilutions of these suspensions were prepared in BAPS and inoculated into suckling 2-day-old mice by i. c. route, to determine the growth of the virus in individual mosquitoes. The titre calculated according to Reed and Muench was expressed as "dex" LD_{50} (haldane, 1960).

Results

Mosquito susceptibility and vector competence

Mosquitoes belonging to all the four species and inoculated with 10^{-1} dilution of CHP virus were tested for the presence of viral antigen at intervals of 12 to 36 hr p. i. None of the mosquitoes tested at 12 and 18 hr were positive. However, 40%, to 80% showed viral antigen at 24 hr (Table 1), then the number of positives at all concentrations increased. The positive rate decreased at higher virus dilutions of the inoculum. The minimum dose required for the detection of CHP antigen in all the three species of *Culex* mosquitoes was 2.5 dex whereas in *Ae. aegypti* it was as low as 0.5 dex. The duration of infection in mosquitoes inoculated with a dilution of 10^{-1} was studied up to day 7 p. i. Over 94% of the mosquitoes tested after 36 hr were found positive.

To determine the vector competence, two *Cx. quinquefasciatus*, six *Cx. tritaeniorhynchus*, eleven *Cx. bitaeniorhynchus*, and eight *Ae. aegypti* mosquitoes were used. *Cx. quinquefasciatus* were fed on a single 2-day-old suckling mouse, while the remaining three species were fed on groups of 4 mice each.

FIG-1

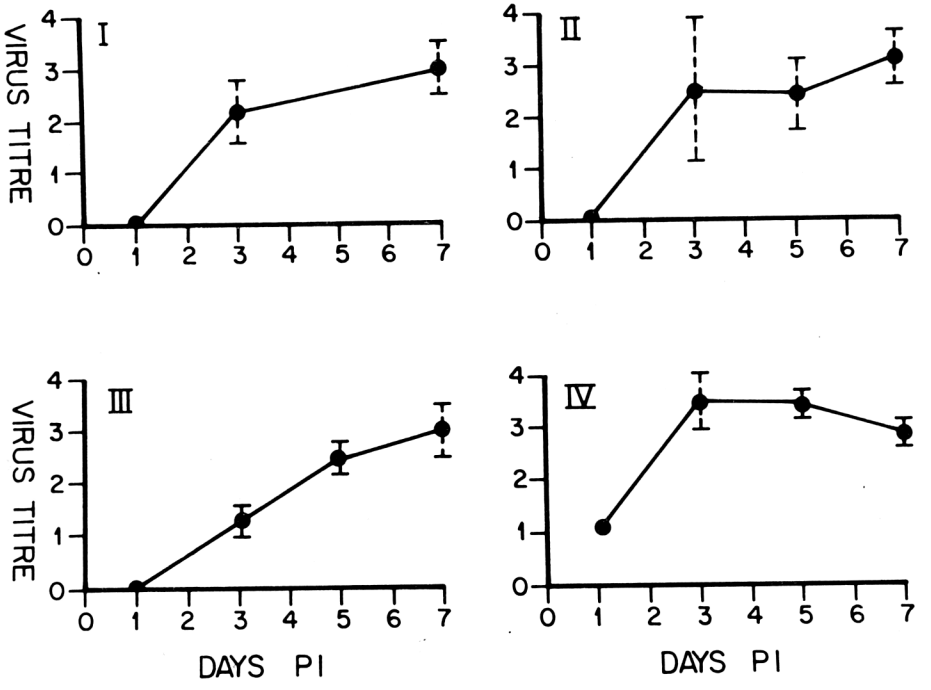

Fig. 1
Growth of Chandipura virus in four species of mosquitoes I. *Culex tritaeniorhynchus;* II. *Cx. bitaeniorhynchus;* III. *Cx. quinquefasciatus;* IV. *Aedes aegypti.*

All the mice became sick and died indicating that all four species of mosquitoes successfully transmitted the virus by bite. The QCF test performed on the brain suspensions of sick mice showed a positive reaction for CHP virus, confirming infection.

Virus growth

The mean virus titre for all four species of mosquitoes obtained in suckling mice is given in Table 2. It ranged between 0.0 to 3.0 dex for *Cx. tritaeniorhynchus* and *Cx. quinquefasciatus*, 0.0 to 3.1 dex for *Cx. bitaeniorhynchus* 1.0 to 3.5 dex for *Ae. aegypti.* The mean titres plotted against time in days clearly showed growth of the virus in all four species of mosquitoes (Fig. 1).

Discussion

In the present study, mosquitoes were infected by parenteral inoculation; about 40 - 80% of mosquitoes inoculated with a dilution of 10^{-1} showed viral

Table 1. Detection of Chandipura virus in mosquitoes inoculated with different virus dilutions and tested at different intervals vy IIFT

Species	*Cx. tritaeniorhynchus*					*Cx. bitaeniorhynchus*					*Cx. quinquefasciatus*					*Ae. aegypti*				
Interval (hours)	12	18	24	30	36	12	18	24	30	36	12	18	24	30	36	12	18	24	30	36
Dilutions*																				
10^{-1}	** 0/5	0/5	4/5	5/5	5/5	0/5	0/5	2/5	5/5	5/5	0/5	0/5	2/5	5/5	4/5	0/5	0/5	3/5	5/5	4/5
10^{-2}	-	-	5/5	5/5	5/5	-	-	3/5	5/5	5/5	-	-	3/5	3/5	5/5	-	-	5/5	5/5	5/5
10^{-3}	-	-	1/5	5/5	4/4	-	-	2/5	5/5	5/5	-	-	1/5	1/5	3/5	-	-	5/5	5/5	5/5
10^{-4}	-	-	0/5	1/5	5/5	-	-	1/5	4/5	3/5	-	-	0/5	2/5	3/5	-	-	5/5	5/5	5/5
10^{-5}	-	-	0/5	1/5	5/5	-	-	0/5	1/5	3/5	-	-	0/5	1/5	0/5	-	-	5/5	5/5	5/5
10^{-6}	-	-	0/5	0/5	0/5	-	-	0/5	0/5	0/5	-	-	0/5	0/5	0/5	-	-	4/5	5/5	5/5
10^{-7}	-	-	0/5	0/5	0/5	-	-	0/5	0/5	0/5	-	-	0/5	0/5	0/5	-	-	1/5	2/5	2/5
10^{-8}	-	-	0/5	0/5	0/5	-	-	0/5	0/5	0/5	-	-	0/5	0/5	0/5	-	-	0/5	0/5	0/5

* The titre of virus in the pool used for serial dilution was 7.5 dex mouse LD_{50}/0.02 ml by i.c. route
** No. positive/No. tested
Note: (-) = Not done

Table 2. Growth of Chandipura virus in four species of mosquitoes after parenteral inoculation

Species		Days post infection			
		1	3	5	7
Culex tritaeniorhynchus	Titre range	0.0	1.5-2.6	ND	2.6-3.4
	Menan titre	0.0	2.2		3.0
	S.D	-	±0.60		±0.56
Culex bitaeniorhynchus	Titre range	0.0	1.5-3.5	1.6-3.1	2.5-3.5
	Mean titre	0.0	2.5	2.4	3.1
	S.D.	-	±1.4	±0.75	±0.59
Culex quinquefasciatus	Titre range	0.0	1.1-1.6	2.2-2.9	2.4-3.4
	Mean titre	0.0	1.3	2.5	3.0
	S.D.	-	±0.25	±0.35	±0.55
Aedes aegypti	Titre range	1.0-1.1	3.0-4.0	3.4-3.5	2.6-2.9
	Mean titre	1.0	3.5	3.4	2.8
	S.D.	±0.05	±0.5	±0.05	±0.17

S.D. Standard deviation
N.D. Not done

antigen at 24 hr p. i. In earlier transmission expreiments (Rao *et al.*, 1969), mosquitoes were infected with CHP virus by feeding on viraemic suckling mice. Even though the infected blood was detected in all the mosquitoes that were tested immediately after feeding, the number that retained the virus varied in different species. While no virus was retained in *Cx. quinquefasciatus*, in other species 25% to 50% of the specimens showed the presence of virus of day 10 p. i. and 25% to 90% of them on day 15 p. i. However, in the present study *Cx. quinquefasciatus* supported multiplication of the virus after parenteral inoculation and transmitted it by bite to suckling mice. Thus, the route on infection seems to be an important criterion in determining the susceptibility of this mosquito species. Therefore, the role of gut barrier, as suggested by Hardy *et al.*(1983) needs to be investigated.

Tesh and Modi (1983) have followed the growth of CHP virus in *Phlebotomus papatasi* by parenteral inoculation. They have shown that the maximum mean titre of 5.6 dex was obtained at 48 hr. In the present study, among *Culex* spp., the maximum titre recorded was 3.5 dex in *Cx. bitaeniorhynctus* followed by 3.4 dex in both *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* on day 7 p. i. In *Ae. aegypti*, the maximum titre of 4.0 dex was observed on day 3 p. i. after which there was a gradual decline.

Since CHP virus has a shorter incubation period of 30 to 36 hr in suckling mice, it is possible to miss the virus, because mortality in suckling mice within a

shorter period can be mistaken as nonspecific. Unlike to mice, in inoculated mosquitoes sickness or death is not the criterion for virus presence which detection is based on the finding of viral antigen. Therefore, mosquito inoculation increases the chances of CHP virus isolation from field specimens.

Among mosquitoes tested during the study, *Ae. aegypti* was the most susceptible. This species can be employed for propagation and detection of CHP virus as a substitute for laboratory mouse if and when necessary.

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