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AMPLIFICATION OF ARBOVIRUS TRANSMISSION BY MOSQUITO INTRADERMAL PROBING AND INTERRUPTED FEEDING

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Summary. — Probing is the crucial phase for the successful intake of the blood by a mosquito female, saliva being ejected during the intradermal probing period. When Ťahyňa virus (California group, family *Bunyaviridae*) carrying and transmitting *Aedes aegypti* mosquito was allowed to feed on 3 suckling white mice for 4 hr, 66.7 % of the 162 exposed mice became infected. When one infected mosquito was put in contact with 5 mice, 53.6 % of the 250 exposed mice became infected. Multiple transmission of the virus to the available hosts during completion of one blood meal by a single mosquito has been demonstrated.

Key words: *arboviruses*; *mosquitoes*; *multiple oral transmission*

Introduction

Crucial phase for the successful intake of the blood by a mosquito female is probing. Mosquitoes search for blood by repeated thrusting of their mouthparts into host's network of deep skin vessels. Saliva is ejected during this intradermal probing period (Griffiths and Gordon, 1952). Successful virus infection of the host seems to correlate with duration of probing and the time of probing depends, at least in part, on the degree of skin vascularization In guinea pigs, mosquitoes located blood faster into the well vascularized ear skin than the poorly vascularized back skin (Ribeiro *et al.*, 1984). The mean duration of blood location of *Aedes aegypti* mosquitoes was 30—40 sec, about half of the mosquitoes failed to locate blood and desisted. Such desisting mosquitoes invariably attempted to refeed (Ribeiro *et al.*, 1985).

Transmission of the virus was observed even after very short probing (Grimstad *et al.*, 1980). Of principal importance for the epiodemiology of any mosquito-borne virus infection is the ability of infected mosquito to transmit the virus to several hosts during completion of a single engorgement. To elucidate this question feeding of *Ae. aegypti* mosquitoes parenterally infected with Ťahyňa virus (California group, family *Bunyaviridae*) on suckling white mice was followed as a laboratory virus — vector — host model.

Materials and Methods

Mosquitoes. *Ae. aegypti* mosquitoes of Indian origin (from National Institute of Virology Pune) with unknown history of collonization were used. Mosquitoes were reared by usual procedures, maintained at the temperature of 27 °C and 75–85 % relative air humidity. Mosquitoes were infected by intrathoracal inoculation of 0.2 µl of Ťahyňa virus mouse brain suspension in the titre of $10^{5.0}$ i.c. mouse LD_{50}/0.01 ml according to the method of Rosen and Gubler (1974) in order to work with 100 % infected mosquitoes. They were provided with swabs soaked into 10 % sugar solution.

Transmission experiments. Seven to eight days post-infection (p.i.) the mosquitoes were separated individually into carton containers (9 cm in diameter and 10 cm height) and the mice were added to allow feeding and virus transfer.

In the first experiment, 3 mice were added to each mosquito for 4 hr. In the second experiment one mouse only was added to each mosquito and the attempts to feed were visually checked. After short intradermal probing period (2 to 5 sec) of each mosquito, the mouse was removed and replaced by another mouse. Each mosquito in the experiment was allowed to probe on three mice.

In the third experiment 5 mice were placed into every carton container containing a single mosquito, again for the time of 4 hr. The experiment was repeated after 7 days with the same surviving mosquitoes (second gonotrophic cycle).

Virus detection. The bitten mice were observed for up to 10 days after the mosquito contact, the brains of mice showing typical signs of illness were used in the next passages. The isolated virus was identified by hyperimmune mouse serum against Ťahyňa virus; the neutralizing index of the serum was 10^6 as tested in i.c. inoculated suckling mice.

Results

In all experiments the transmission rate varied from 62.3 % to 71.4 % (mosquitoes transmitting virus/mosquitoes used × 100). When three mice were brought in contact with the infected mosquito the transmission rate was 71.4 %. Only one out of the three exposed mice became infected by 14 out of 56 mosquitoes in the experiment (25.0 %). Two out of three mice became infected by 12 mosquitoes (21.4 %) and to all three mice the virus was transferred by 14 mosquitoes, which represented 25.0 % (Table 1). In another experiment only short three subsequent probings for 20 to 30 min of 20 mosquitoes were visually checked; the transmission rate was 70 %. Five mosquitoes transferred the virus to only one out of three mice and

Table 1. Results of the oral transmission of Ťahyňa virus by Ae. aegypti individually kept together with three baby mice

No. of mice infected/ in contact with the mosquito	Mosquitoes tested	
	A. Contact for 4 hr	B. Probing only
0/3	16 (28.6 %)	6 (30 %)
1/3	14 (25.0 %)	5 (25 %)
2/3	12 (21.4 %)	4 (20 %)
3/3	14 (25.0 %)	5 (25 %)

A. All three mice exposed to the mosquito bite at once (56 mosquitoes tested)
B. Mosquitoes allowed only to probe on each of the three mice (20 mosquitoes tested)

Table 2. Results of the oral transmission of Ťahyňa virus with Ae. aegypti individually kept together with five baby mice for 4 hr in two subsequent transmissions

No. of mice infected/ in contact with the mosquito	Mosquitoes tested in	
	1st gonotroph. cycle	2nd gonotroph. cycle
0/5	20 (37.7 %)	10 (37.1 %)
1/5	11 (20.8 %)	2 (7.4 %)
2/5	8 (15.1 %)	5 (18.5 %)
3/5	5 (9.4 %)	4 (14.8 %)
4/5	2 (3.8 %)	5 (18.5 %)
5/5	7 (13.2 %)	1 (3.7 %)

another 5 to all three mice, respectively (25 % each). Four mosquitoes transferred virus to two mice, amounting 20 % (Table 1). In both experiments mentioned above, 162 suckling mice were in contact with 54 mosquitoes that transmitted virus and 108 mice became infected (66.7 %).

The contact of mosquitoes with five mice was performed in two subsequent gonotrophic cycles. In the first feeding 53 intrathoracally infected mosquitoes were tested with a total transmission rate of 62.3 %. Only to one out of five mice transferred the virus 11 mosquitoes (20.8 %), to two mice the virus was transmitted by 8 mosquitoes (15.1 %), to three by 5 (9.4 %) and to four mice it was transmitted by 2 mosquitoes (3.8 %). All five mice became infected after being in contact with 7 individual mosquitoes placed into 7 different containers (13.2 %).

In the second blood feeding 27 surviving mosquitoes were tested; the transmission rate was 62.9 %. Only one mouse became infected by 2 mosquitoes (7.4 %). Two mice were infected by 5 mosquitoes (18.5 %), three mice by 4 (14.8 %), four mice by 5 (18.5 %) and all five mice became infected by 1 mosquito, comprising 3.7 % of mosquitoes in the second gonotrophic cycle. (Table 2).

In the latter experiment together 250 baby mice were in contact with the transmitting mosquitoes and 134 mice became infected. After the first feeding 33 mosquitoes transferred virus to the 85 experimental animals (51.5 %). During the second blood feeding 17 mosquitoes transmitted virus to 49 mice, which represented 57.6 % of all 85 mice being in contact with the transmitting mosquitoes. In these two subsequent blood feedings one mosquito transferred virus to all 10 mice, three another to 9 baby mice each.

Discussion

It has been known that the mosquito can transfer virus when probing (Chamberlain and Sudia, 1961; Grimstad *et al.*, 1980). According to recent facts mosquitoes salivate (almost) exclusively during the probing phase, which is connected with the function of the saliva in the mosquito blood-feeding.

The saliva contain the enzyme apyrase with antihaemostatic activity helpful in finding the source of the blood in the host skin (Ribeiro, 1987). Our experiments demonstrated multiple transmissions of the virus to the available hosts. The results of the laboratory experiments with unnatural vectors usually have a preliminary value only. But the observed amplification of the oral transmission of Ťahyňa virus with *Ae. aegypti* mosquitoes is not virus-specific, nor mosquito-specific and is connected with salivation and blood-feeding behaviour of mosquitoes. From this point of view we have to reexamine the circumstances under which different mosquito-borne viruses are orally transmitted. Of importance are detail informations on the salivation and probing activity of mosquito vectors. Various feeding strategies of mosquitoes might be involved as the result of evolutionary trends inside the family *Culicidae* (Gillett, 1967). In the light of the presented results "nervous" mosquito species, making many probes to complete a blood-feeding, are more important vectors. Moreover, the importance of every transmitting mosquito under natural conditions is much higher and the importance even of the low rate transovarial transmission of the virus in mosquitoes is stressed.

The rate of probing influenced also the behaviour of hosts. Multiple feeding of *Ae. aegypti* mosquitoes in a laboratory experiment on the arms of two human volunteers was significantly increased when both moved their arms (Lenahan and Boreham, 1976). Higher mosquito densities caused more defensive activity of experimental birds and consequently more mosquitoes were interrupted during feeding (Edman *et al.*, 1972). Reeves (1971) suggested that large vector populations could cause avian hosts to become increasingly defensive with a subsequent diversification of feeding to more aberrant mammalian hosts including man.

Edman and co-workers found that differences in the grooming intensity of ciconiiform birds were associated with differences in the level of mosquito feeding success (Edman and Kale, 1971; Edman *et al.*, 1972). That frequency of multiple feeding may have an important impact on the epidemiology of several mosquito-borne diseases (Klowden and Lea, 1978; Mitchell and Millian, Jr., 1981) was previously suggested.

The infection of mosquitoes with different pathogens can also influence their feeding behaviour. Ribeiro *et al.* (1985) stated that transmission of parasites would be enhanced by prolonged probing, caused either by deficiency of antihaemostatic components of saliva or by obscuring phagoreceptors. In the case of an avian malaria like *Plasmodium gallinaceum*, median blood location time by sporozoite-infected mosquitoes is three times longer than that by noninfected mosquitoes (Rossignol *et al.*, 1984). *Rhodnius prolixus* locate blood with reduced effectiveness when infected with *Trypanosoma rangeli* (Anez and East, 1984).

The evidence has been accumulated, that arboviral infection enhanced the probing activity of mosquitoes. *Ae. triseriatus* females infected with La Crosse virus exhibited a significant tendency to probe more frequently than did uninfected siblings (Grimstad *et al.*, 1980). This is one more data to stress

the importance of the observed amplification of the oral transmission with mosquitoes in the circulation of any mosquito-borne arbovirus in nature.

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