EXPERIMENTAL INFECTION OF *ANOPHELES ALBIMANUS* AND *CULEX THRIAMBUS* MOSQUITOES WITH VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS TC-83 STRAIN

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ABSTRACT. *Anopheles albimanus* and *Culex thriambus* mosquitoes were infected with Venezuelan equine encephalomyelitis virus, TC-83 strain, by allowing them to feed on suckling mice inoculated with virus and by intrathoracic mosquito inoculation. Transmission of the virus to one suckling mouse occurred using *An. albimanus* mosquitoes after a 10-day extrinsic incubation period. The results are discussed.

The pathogenicity of Venezuelan equine encephalomyelitis (VEE) virus for equines and laboratory animals has been modified through serial passages in primary cell cultures of fetal guinea pig heart (Berge et al. 1961). The TC-83 strain was obtained and it is used for vaccination of equines in enzootic areas of Latin America; however, there is the possibility that the virus might infect mosquitoes and they may spread the virus in nature.

Under laboratory conditions it has been possible to infect *Aedes aegypti* and *Ae. triseriatus* mosquitoes with TC-83 strain of the VEE virus (Schaffer and Scherer, 1971, 1974). In field conditions, Pedersen et al. (1971) were able to isolate TC-83 strain virus from *Psorophora confinnis* mosquitoes. However, other workers have not been able to isolate vaccinal virus from mosquitoes living in areas where equines have been vaccinated previously, suggesting that the titer of the viremia in these animals was too low to infect mosquitoes (McConell 1971; Walton et al. 1972).

In the present study, it was possible to infect *Anopheles albimanus* and *Culex thriambus* with the TC-83 strain under laboratory conditions and in one instance it was possible to transmit the virus to a suckling mouse.

MATERIALS AND METHODS. The virus used was the TC-83 strain of VEE, passage 85, in primary cell culture of fetal guinea pig heart. The titer was $10^{8.0}$ LD_{50}/suckling mouse/intracerebral/milliliter (LD_{50}/sm/ic/ml).

The *An. albimanus* mosquitoes came from our colony in the laboratory. The *Cx. thriambus* mosquitoes were obtained in the pupal stage from the area surrounding the Instituto Nacional de Investigaciones Pecuarias, México, D.F. and were maintained in the laboratory until they reached the adult stage.

In the first experiment, *An. albimanus* were exposed by allowing them to feed for 30 min on suckling mice inoculated with TC-83 strain of VEE virus. These animals showed clinical signs of encephalitis, and they had a viremia titer of $10^{7.2}$ LD_{50}/sm/ic/ml. After feeding, the mosquitoes were separated in cardboard cups and maintained at a temperature of 28°C and 70-80% R.H. During the extrinsic incubation period the mosquitoes were fed daily with cotton saturated with a 5% sterile sugar solution. At 0, 3, 7, 8, 9, 10, 11, 12 and 13 days after infection, pools of 3 to 7 mosquitoes were ground in a mortar with diluent prepared with Hank's Balanced Salt Solution (HBSS), 25% decomplemented normal rabbit serum, 1.6 mg of streptomycin and

1,000 U of penicillin per ml (Sudia and Chamberlain 1967). The suspensions were centrifugated at 1,700 x g, 30 min at 4°C and the supernatant was inoculated by the IC route into suckling mice of 1 to 3 days of age. The inoculums in which virus was detected were titrated in suckling mice according to the method of Reed and Muench (1938).

In a second experiment, mosquitoes that had been exposed 10, 11, 12, and 13 days earlier were allowed to feed for 30 min on normal suckling mice 1 to 3 days old. The transmission of virus by mosquitoes in these animals was confirmed by the virus-neutralization test using a hyperimmune serum prepared in rabbit, with a hemagglutination-inhibition titer of 1:640.

In the third experiment, *Cx. thriambus* mosquitoes were anesthetized at −20°C for 5 minutes and they were inoculated by the intrathoracic route with approximately 0.001 ml of a virus suspension with a titer of $10^{5.0}$ LD_{50}/sm/ic/ml. The inoculation was done with a sterile microsyringe made from a capillary tube, according to the technique described by Rosen and Gubler (1974). The inoculated mosquitoes were separated in cardboard cups and maintained at a temperature of 28°C and relative humidity of 70–80%. They were fed daily with a 5% sterile sugar solution. Sixteen, 23, 26, 36 and 43 days after the inoculation, mosquito suspensions were prepared and titrated in suckling mice, as described above.

Table 1. Infectivity of Venezuelan equine encephalomyelits virus, TC-83 strain, for *Anopheles albimanus* mosquitoes.

Day post-infection [a]	Number of mosquitoes in each group	Virus in the pool of mosquitoes	Average virus titer per mosquito [b]
0	7	yes	NT [c]
3	6	—	..
7	5	yes	$10^{3.3}$
8	5	yes	$10^{4.3}$
9	4	yes	$10^{4.3}$
10	5	yes	$10^{7.3}$
11	4	yes	$10^{5.2}$
12	6	yes	$10^{3.9}$
13	3	yes	$10^{4.2}$

[a] The mosquitoes were infected by allowing them to feed on inoculated suckling mice for 30 min. The mosquitoes were separated and maintained at 28° C and a relative humidity of 70–80%.

[b] Virus titer expressed as LD_{50}/sm/ic/ml.

[c] Not tested.

RESULTS. In the first experiment, most of the mosquitoes became infected by feeding on mice with a viral titer of the blood of $10^{7.2}$ LD_{50}/sm/ic/ml. The average titer of virus per mosquito as LD_{50}/sm/ic/ml was as follows: at 0 days, virus was detected but it was not titrated; 3 days after infection it was not possible to isolate virus; at 7 days, the titer was $10^{3.3}$; at 8 days, $10^{4.3}$; at 9 days, $10^{4.3}$; at 10 days, $10^{7.3}$; at 11 days, $10^{5.2}$; at 12 days, $10^{3.9}$ and at 13 days the viral titer was $10^{4.2}$ (Table 1).

In the second experiment, the mosquitoes exposed 10 days earlier to infection were able to transmit the virus to one suckling mouse of 6 exposed. The animal died at the 5th day of exposure; the virus could be isolated and neutralized with a specific VEE antiserum (Table 2).

In the third experiment, the intrathoracic inoculation of *Cx. thriambus* was successful. The average titers per mosquito expressed in LD_{50}/sm/ic/ml were as follows: at 16 days post-inoculation, $10^{5.9}$; at 23 days, $10^{8.0}$; at 26 days, $10^{6.3}$; at 36 days, $10^{7.7}$ and at 43 days, $10^{4.9}$ (Table 3).

DISCUSSION. According to the results, TC–83 strain of VEE virus is able to easily infect *An. albimanus* and *Cx. thriambus* mosquitoes. Thus, the pathogenicity of the VEE virus has been modified for equines and laboratory animals, but it retains the ability to infect and grow in mosquitoes as has also been demonstrated by Schaffer and Scherer (1971, 1974) in *Ae. aegypti* and *Ae. triseriatus*.

Table 2. *Anopheles albimanus* mosquito transmission of Venezuelan equine encephalomyelitis virus, TC-83 strain, to suckling mice.

Day post-infection[a]	Number of mosquitoes in each group	Dead / exposed mice	Virus in the pool of of mosquitoes	Average virus titer per mosquito[d]
10	5	1/6[be]	yes	$10^{7.3}$
11	4	0/6	yes	$10^{6.2}$
12	6	0/6	yes	$10^{3.9}$
13	3	0/6	yes	$10^{4.2}$

[a] The mosquitoes were infected by allowing them to feed on inoculated suckling mice for 30 min. The mosquitoes were separated and maintained at 28° C and 70–80% R.H.
[b] Normal suckling mice were exposed by allowing the group of infected mosquitoes to feed on them for 30 min. All the mosquitoes took a blood meal from at least 2 of the 6 suckling mice exposed.
[c] VEE virus demonstrated by the virus-neutralization test using hyperimmune serum.
[d] Virus titer expressed as LD_{50}/sm/ic/ml.

The growth of the virus followed the pattern of other Togaviruses of the Group A. In the transmission experiments it is noteworthy that only in one case was transmission successful and only in one animal. The viral titer reached for mosquitoes of that group was high when compared with those unable to transmit the virus. These results are similar to those reported by Collins et al. (1963, 1965) and Collins and Harrison (1966) with *An. albimanus* where mosquitoes became infected easily with other arboviruses but transmission ability in this species was low. To be sure that the virus transmitted by these mosquitoes to the suckling mouse was VEE, a virus-neutralization test and a 2nd passage was done confirming the results.

Table 3. Experimental infection of *Culex thriambus* mosquitoes with Venezuelan equine encephalomyelitis virus, TC-83 strain, by intrathoracic inoculation.

Number of inoculated mosquitoes[a]	Days post-inoculation	Virus in the pool of mosquitoes	Average virus titer per mosquito[b]
6	16	yes	$10^{5.9}$
1	23	yes	$10^{8.0}$
3	26	yes	$10^{6.3}$
2	36	yes	$10^{7.7}$
1	43	yes	$10^{4.9}$

[a] The mosquitoes were anesthetized at −20° C for 5 min and inoculated by intrathoracic route with a microneedle, with approximately 0.001 ml of virus of a titer of $10^{5.0}$ LD_{50}/sm/ic/ml.
[b] Virus titer expressed as LD_{50}/sm/ic/ml.

The viremia titer of inoculated suckling mice was $10^{7.2}LD_{50}$/sm/ic/ml and it was enough to successfully infect the mosquitoes. Usually the viremia titers reached in vaccinated horses is of $10^{1.9}$ LD_{50}/sm/ic/ml which is insufficient to infect *Ae. triseriatus* (Sudia et al. 1971). In nature on only one occasion has TC–83 strain been isolated from *Ps. confinnis* mosquitoes (Pedersen et al. 1971) indicating that the threshold of viremia titer for mosquito infection varies according to the species. Therefore, there is the possibility that *An. albimanus* and *Cx. thriambus* may become infected from the vaccinated horses. This is a subject that requires further research. The possibility that mosquitoes may become infected with TC–83 strain under field conditions and spread the virus in nature has to be considered. In Mexico, this is important due to the fact that there is a national equine vaccination campaign and it is necessary to be sure that the virus is not spreading in nature. However, there is no indication that virus is spreading to other domestic animals (McConell 1971, Walton et al. 1972, Gallo et al. 1976) demonstrating that the TC–83 vaccinal strain is safe

under field conditions, despite its growth in mosquitoes.

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