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ISOLATION OF ŤAHYŇA VIRUS FROM MOSQUITOES *CULISETA ANNULATA* (SCHRK. 1776)

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Abstract. A description is given of the first isolation of Ťahyňa virus from mosquitoes *Culiseta annulata* (Schrk. 1776) from nature. The isolation was performed from a suspension prepared from 18 mosquitoes of this species collected towards the end of September 1968 in the cave Turold near Mikulov in southern Moravia. The difference was recorded between the subcutaneous susceptibility of suckling mice to the freshly isolated strain and their susceptibility to high passage of intracerebrally passaged strain of Ťahyňa virus. In higher intracerebral passages the subcutaneous susceptibility is minimal.

We have experimentally demonstrated in previous papers that Ťahyňa virus multiplies in the mosquitoes *Culiseta annulata* (Schrk. 1776), that it can be transmitted by them to the host and that it can hibernate in them (DANIELOVÁ, MINÁŘ, ROSICKÝ 1968; ŠIMKOVÁ and DANIELOVÁ 1969; DANIELOVÁ and MINÁŘ 1969). In order to make sure that the species *C. annulata* actually takes part in the circulation of the virus in nature and especially in its hibernation, we performed isolation tests from these mosquitoes, partly in winter, i.e. from the hibernating mosquitoes, and partly in autumn, i.e. immediately before hibernation.

MATERIAL AND METHODS

The collecting of mosquitoes was carried out in southern Moravia, in the wide neighbourhood of Mikulov. The first collecting was carried out in February 1968 in the localities of Drnholec, Jevišovka, Pálava (Mikulov), Turold, Lednice and Valtice. Before processing the mosquitoes were kept for 11 to 14 days in thermostat at 18.5—22 °C and 60—90 % relative humidity. In the second phase the mosquitoes *C. annulata* were collected at the end of September 1968 at Turold and Nejdek. Until processing, i.e. for 48 days they were kept on dry ice.

Isolation tests: 8—18 mosquitoes (on an average 15, in one case 2) were triturated in 1 ml of 10 % inactivated normal calf serum in saline pH 7.4 with 1000 units PNC and 1000 γ STM. The suspension was placed in refrigerator for 30—60 minutes and thereafter centrifuged for 10 min. at 2500 rev./min. The supernatant was inoculated into 1 litter of 2—4 day-old suckling mice in the

dose of 0.01 ml i. cer. and 0.03 ml s. cut. per mouse. The mice were observed for 2 weeks, the brains of dead and sick mice were passaged in suckling mice as above.

Identification of virus was done by neutralization test. The mixture of 10-fold dilutions of 20 % mouse brain suspension and mouse hyperimmune serum anti Ťahyňa was incubated for 90 min. at 37 °C and inoculated into juvenile 8—10 g mice in the dose of 0.02 ml i.cer. per mouse. The control strain 181 of Ťahyňa virus was isolated from mosquitoes *Aëdes vexans* in southern Moravia (KOLMAN et al. 1964).

Titration of the isolated strain was performed in 2—3 day-old suckling mice by inoculation of 10-fold dilutions in the dose of 0.01 ml i. cer. as well as 0.03 ml s. cut. per mouse and in 8 g mice by inoculation of the dose of 0.02 ml i. cer. or 0.06 ml s. cut. per mouse.

Isolation of the virus was carried out in the field laboratory at Valtice in southern Moravia, where the virus Ťahyňa was not studied at that time.

RESULTS

During the isolation tests performed in February 1968 a total of 222 mosquitoes *C. annulata* were dealt with in 15 isolation tests. The results were negative.

In isolation tests in the autumn only 20 mosquitoes *C. annulata* were dealt with in 2 isolation tests. From the isolation mixture No. 5079 prepared from 18 mosquitoes *C. annulata* collected in the cave Turold near Mikulov one strain of virus was isolated. The re-isolation from initial material could not be performed for lack of material. The virus was identified by neutralization test as virus Ťahyňa. In the neutralization test prepared from third passage brain suspension the neutralization index reached the value 4.0 log LD_{50}, in the repeated test from lyophilisate of third passage brain suspension the value 4.5 log LD_{50}.

By titration of fresh brain suspension on the one hand and of the lyophilisate of third passage brain suspension on the other we verified the susceptibility of

Table 1. Susceptibility of mice to strains 5079 and 181

Material	Suckling mice		Juvenile mice	
	i.cer.titre*)	s.cut.titre*)	i.cer.titre*)	s.cut.titre*)
Strain 5079 Brain suspension of third passage	> 8.43	> 7.50	7.83	—
Strain 5079 Lyophylisate of third passage	7.62	6.70	6.50	negative
Strain 181 Lyophylisate of brain suspension of 56th passage	5.84	< 2.00	—	—

*) expressed in log LD_{50}.
– was not performed.

suckling and juvenile mice to this strain. Tab. 1 shows relatively high titre values of this strain and for comparison also presents values for strain 181.

As apparent from the table, the strain 5079 shows a striking difference in higher subcutaneous susceptibility of suckling mice. The difference is caused primarily

Table 2. Comparison of virus titre in low and high passages of virus Ťahyňa strains 181 and 92*

Material	Titre i.cer. in log LD_{50}	Titre s.cut. in log LD_{50}
Strain 181 3rd passage	7.62	5.70
Strain 181 52nd passage	6.75	0.55
Strain 92 low passage**	7.00	4.70
Strain 92 32nd passage	6.60	0.55

*) Strain isolated from mosquitoes *Aëdes caspius* in eastern Slovakia (Bárdoš, Danielová 1959).

**) Data given by Bárdoš, Čupková and Šefčovičová (1961) apply to 4th—14th passage.

by different passage of both strains. We ascertained that subcutaneous susceptibility of suckling mice is diminishing in higher passages of the intracerebrally passaged virus Ťahyňa. The difference is presented in Table 2. The high subcutaneous suceptibility may serve as evidence of the freshly isolated strain and can eliminate the confusion with contamination by laboratory strain of Ťahyňa virus.

DISCUSSION

The isolation of Ťahyňa virus from mosquitoes *C. annulata*, in which the virus multiplies as previously demonstrated and can be transmitted to a healthy host (Danielová, Minář, Rosický 1968; Danielová, Minář 1969; Šimková, Danielová 1969), gives evidence that this mosquito species in nature participates in the circulation of Ťahyňa virus as vector.

From the epidemiological aspect its importance cannot be great because it occurs very rarely and consequently does not probably play a considerable role in the dissemination of the virus during vegetation period. Its sporadic occurrence is also the reason why a low number of mosquitoes was dealt with in isolation tests. Its importance, however, rests with its capability of maintaining the virus for a period between autumn and spring and of creating a link between the last year's and this year's population of vectors from the genus *Aëdes*, especially of the species *Aëdes vexans*, which propagates dissemination of the virus in nature. As demonstrated previously (Danielová, Minář 1969), the virus is maintained in this mosquito as long as 181 days between autumn and spring and its amount does not diminish.

REFERENCES

Bárdoš V., Čupková E., Šefčovičová L., The Ťahyňa virus II. Characteristics of some biological properties and preliminary immunological classification. Acta virol. 5: 93 to 100, 1961.

—, Danielová V., The Ťahyňa virus—a virus isolated from mosquitoes in Czechoslovakia. J. Hyg. Epidem. (Praha) 3: 264—276, 1959.

Danielová V., Minář J., Experimental hibernation of Ťahyňa virus in mosquitoes *Theobaldia annulata* (Schrk.) (Diptera, Culicidae). Folia parasitol. (Praha) 16: 285—287, 1969.

—, —, Rosický B., Experimental survival of the virus Ťahyňa in hibernating mosquitoes *Theobaldia annulata* (Schrk.). Folia parasitol. (Praha) 15: 183—187, 1968.

Kolman J. M., Málková F., Nemec A., Smetana A., Hájková Z., Minář J., The isolation of the Ťahyňa virus from the mosquito *Aëdes vexans* in Southern Moravia. J. Hyg. Epidem. (Praha) 8: 380—385, 1964.

Šimková A., Danielová V., Experimental infection of chimpanzees with Ťahyňa virus by *Theobaldia annulata* mosquitoes, Folia parasitol. (Praha) 16: 255—263, 1969.

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