

## ISOLATION OF ČALOVO VIRUS FROM *ANOPHELES MACULIPENNIS* S. L. MOSQUITOES IN YUGOSLAVIA

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**Abstract.** The authors report on the first isolation of the virus Čalovo (Bunyamwera group) in Yugoslavia. The virus was isolated from mosquitoes *Anopheles maculipennis* s.l. collected in the village Marino Selo (Croatia).

In the summer of 1969 the first virological testing of mosquitoes in Croatia was performed jointly by the Virus Department, Andrija Štampar School of Public Health, Medical Faculty, University of Zagreb, and the Institute of Parasitology of the Czechoslovak Academy of Sciences, Prague. The tests were initiated by the finding of antibodies against the Ťahyňa virus in forest workers in the north-east of Croatia.

### MATERIAL AND METHODS

Isolation experiments were performed in baby mice (2–3 days old) by the intracerebral (0.01 ml) and subcutaneous (0.03 ml) inoculation of the mosquito suspensions. The suspensions were prepared by grinding the mosquitoes in the saline, pH 7.4, containing 10% inactivated calf serum, 500 units of penicillin, and 500 gamma of streptomycin in 1 ml.

In the neutralisation tests two strains of the Čalovo virus were used as antigens: strain 134 isolated from mosquitoes in South Moravia, Czechoslovakia (SMETANA et al. 1967) in 1963 and strain 184—the original strain of the virus Čalovo isolated in South Slovakia, Czechoslovakia (BÁRDOŠ, ČUPKOVÁ 1962) in 1960. The mice hyperimmune sera used were anti Čalovo 184, anti Ťahyňa 181, and anti new strain MS 3. The tests were performed in 8 g white mice.

### RESULTS

A total of 20,486 mosquitoes were captured and tested. Out of this number 10,320 mosquitoes belonged to the species *Anopheles maculipennis* s.l. Of the 52 pools of 200 mosquitoes each, prepared from this species, 10 strains of infectious agents have been isolated so far. The first strain MS 3 isolated from mosquitoes captured in a shed of the village Marino Selo was reisolated from the original suspension stored at –70 °C and successfully passaged. The incubation period in the primoinoculation was 6–8 days, in the second passage 3–5 days, and in the third passage 3–4 days.

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The strain was identified in the neutralisation test with antisera against the Čalovo and Ťahyňa viruses. The result is shown in Table 1. After preparing the antiserum against the new MS3 strain the cross-neutralisation test with the original

**Table 1.** The neutralisation test with Čalovo and Ťahyňa antisera

Virus	Serum	
	anti Čalovo 184*)	anti Ťahyňa 181
MS3	3.84	0
Čalovo 134	> 5.00	—

\*) in log NI.  
— not done.

**Table 2.** The cross-neutralisation test with Čalovo strain 184

Virus	Serum	
	anti MS3*)	anti Čalovo 184*)
MS3	≥ 5.05	4.84
Čalovo 184	4.83	5.83

\*) in log NI.

Čalovo virus strain 184 and its antiserum was performed. The result is seen in Table 2.

In view of the results presented we consider the new isolated MS3 strain almost identical with the original virus Čalovo (the Bunyamwera group) strain 184. The isolation of the Čalovo virus from mosquitoes is the first isolation of the virus in Yugoslavia.

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