

## ISOLATION OF ŤAHYŇA VIRUS FROM BITING MIDGES (DIPTERA, CERATOPOGONIDAE) IN CZECHO-SLOVAKIA

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**Summary.** – A total of 14 250 haematophagous biting midges (genus *Culicoides*, Diptera: *Ceratopogonidae*) were collected in June 1986 in the mountains Českomoravská vysočina (about 600 m above sea level); from these 2 strains of Ťahyňa virus (serogroup California, *Bunyaviridae*) were isolated. To our knowledge this is the first isolation of a California serogroup virus from the species of family *Ceratopogonidae* as well as the first report of arbovirus isolation from biting midges in Europe.

**Key words:** *Ceratopogonidae*; haematophagous species; Ťahyňa virus; isolation

### Introduction

Bloodsucking representatives of the family *Ceratopogonidae* (Diptera) are since many years known as undesired ectoparasites attacking wild living and domestic animals, birds, as well as man. In warm regions they contribute to transmission of pathogens such as haemosporidia, microfilariae, and last but not least of viruses.

More than 60 arboviruses can be transmitted by biting midges largely from genus *Culicoides*, including agents such as bluetongue, African horsesickness, Eastern equine encephalitis, Nairobi sheep disease, Congo haemorrhagic fever, Oropouche fever, and others. With many other viruses their association to clinical disease has been anticipated. Because of the lack of investigation of bloodsucking biting midges as viral vectors in Czechoslovakia, we decided to evaluate the role of Diptera in arbovirus transmission.

### Materials and Methods

Specimens were collected in sites surrounding the town Telč (Jihlava district, South Moravia). The investigated locality is situated in the mountains Brtnické vrchy, 470 – 712 meters above sea

level. The mean temperature there is  $6.5^{\circ}\text{C}$ , the total average precipitation 617 mm, the growing season lasts for 143 days (Vesecký, 1961). Geologically the area belongs to moldanubicum; it is covered with mesophytic vegetation of submountain level (Fagion). The small rodents are frequent (*Sorex araneus*, *Neomys fodiens*, *Mus musculus*, *Apodemus flavicollis*, *Apodemus sylvaticus*, *Microtus arvalis*, *Clethrionomys glareolus*; Homolka, personal communication). From larger mammals *Lepus europeus*, *Vulpes vulpes*, *Meles meles*, *Sus scrofa* can be found, less frequently *Oryctolagus cuniculus* can occur. From large mammals *Capreolus capreolus*, *Dama dama*, and *Cervus elaphus* are worth mentioning (Zima, personal communication). The land is covered with forests or fields, ponds are frequent. Grazing of cattle is regular, occasionally goats may be seen. The areas of specimen collection were close to water surface with exception of locality II (Lipky). Material was collected in 5 localities, mostly in locality I.

Locality I ("Stará Říše - Pilka") is a clearing 605 - 610 above sea level about 300 m from a larger forest edge (spruce with occasional pines and a few deciduous trees). Nearby (50 m) is a pond ("Vodnatý"). Locality II is called "Lipky". Locality III is called "Lhotka" at the pond "Svatojánský". Locality IV "Řásná" is at pond "Velkopařezý" and locality "Řídelov" at the pond "Pílný".

The imagoes were captured by the help of an entomologic net at attacking men (about 90% of biting midges) or they were collected from sentinel rabbits or by a light trap. From June 23 to June 26, 1986 altogether 14 250 imagoes and females from genus *Culicoides* were captured. Seven species were identified (listed according to their frequency): *Culicoides obsoletus* (Meigen, 1918), *C. impunctatus* (Goetghebuer, 1920), *C. achrayi* (Kettle and Lawson, 1955), *C. pallidicornis* (Kieffer, 1919), *C. pulicaris* (Linne, 1758), *C. heliophilus* (Edwards, 1921), and *C. griseus* (Edwards, 1939). The spectrum of species was estimated by identification of 1505 biting midges, a sample regarded for representative.

Samples destined for virus isolation were shipped in glas tubes on dry ice and then stored at  $-70^{\circ}\text{C}$  until examined. SPF outbred suckling mice (1-3 days old) from breed Velaz (Prague) were inoculated by intracerebral (i.c.) route. The suspensions from 250 imagoes were prepared in 1.5 ml L-15 medium containing 3% foetal calf serum and antibiotics; they were clarified by centrifugation (1500 rev/min, 15 min) and their sterility was checked on meat-peptone and thioglycollate broth incubated for 5 days at  $37^{\circ}\text{C}$ . The infected mice were observed for 20 days.

The brains from dead and sick mice were homogenized and inoculated into suckling mice until 100% lethality was achieved. The passaged isolates were examined for their physicochemical and biological properties. The tests included: 1. membrane filtration at 100 nm and 220 nm (Millipore filters) as described by Hsiung (1965) and Casals (1968); 2. determination of sensitivity to diethylether (Andrews and Horstmann, 1949), and/or to sodium deoxycholate (Lenette *et al.*, 1969); 3. pathogenicity for suckling and adult mice by i.c., intraperitoneal (i. p.), and subcutaneous (s.c.) routes. CPE and plaque formation were tested in Vero and PS cells; infectivity titrations were made in suckling mice and in Vero cells the  $\text{LD}_{50}$  and  $\text{TCID}_{50}$  values being calculated according to Reed and Muench (1938).

The virus isolates were identified in virus-neutralization tests by micromodification of plaque reduction test (de Madrid and Porterfield, 1969) in Vero cells with immune sera to following viruses: Lipovnik, Ťahyňa, Tribeč and subtype Brezová (Hubálek *et al.*, 1988), Uukuniemi, Bhanja, Eyach, Čalovo, Sindbis, West Nile, tick borne encephalitis virus, and vesicular stomatitis virus. Several Ťahyňa virus strains isolated in the ČSFR were used: the prototype strain "92" (Bárdoš and Danielová, 1959) strain "236" (Bárdoš *et al.*, 1961), strain "La65" (Bárdoš *et al.*, 1975b), strains "T16" and "P6b" (Bárdoš *et al.*, 1975a).

### Results

Two viruses designated C/T38 and C/T39 were isolated from unengorged females collected in the locality I from the pools containing *C. obsoletus*, *C. impunctatus*, *C. achrayi*, *C. pallidicornis*, *C. pulicaris*, *C. heliophilus*, and *C. griseus*, the first species dominating at 71.2%. Both agents passed the 220 nm filters but not the 100 nm ones. Diethylether and SDC decreased the titre of isolates by 5 log as measured in Vero cells. The isolated viruses developed CPE in Vero as well as in PS cells. Their titre in Vero cells reached  $10^{-9}$  and clearcut plaques were formed in these cells. Both isolates were pathogenic for suckling and adult mice by i. c. inoculation and for suckling mice by s. c. and i. p. inoculations. Peripheral inoculation was not lethal for adult mice. The infectious titre in suckling mice has reached  $10^{-10.1}$  LD<sub>50</sub>/ml.

Plaque reduction neutralization on microplates with a panel of antisera (*Materials and Methods*) showed that the isolates are identical with Ťahyňa virus. Basic estimations were made by 80% plaque reduction. For calculations the C<sub>7</sub> coefficient method was used (Table 1) (Hubálek, 1982). Cluster analysis expressed in the dendrogram (Fig. 1) shows the higher antigenic relatedness of our isolate to strains La65, T 236 and P6b than to T16 and 92.

### Discussion

Isolation of bunyavirus Ťahyňa (*Bunyaviridae*, genus *Bunyavirus*, group *Bunyamwera*, serogroup *California*) known in Europe, Asia, and Africa (Bárdos, 1974) is in Europe first reported from biting midges, members of

Table 1. Crosstiteration by plaque reduction of the 6 Ťahyňa virus strains with corresponding antisera\*

Virus	C/T38	P6b	T16	Serum		
				La65	236	92
C/T38	10.5**	9	8	9	9	7.5
P6b	9	8.5	7.5	8.5	8.5	5.5
T16	7	7	7.5	7	7	4.5
La65	8	7	6.5	7.5	7	5
236	7.5	6.5	5.5	7	6.5	4.5
92	8.5	8.5	7	8.5	9	8

\* three immunization doses in mice

\*\* log<sub>2</sub> of titre reciprocals

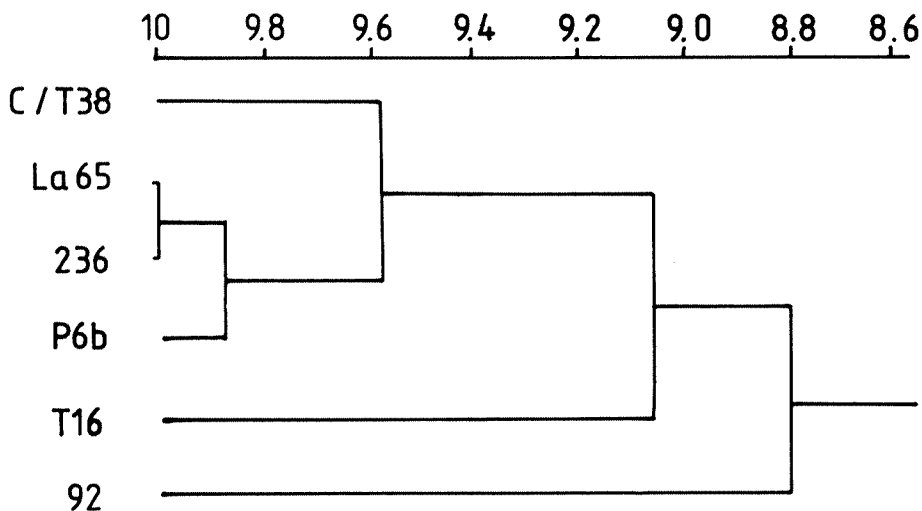


Fig. 1

Dendrogram depicting the antigenic relatedness among 6 Tăhyňa virus strains (based on the data from Table 1) using the  $C_7$  coefficient and cluster analysis (UPGMA)

genus *Culicoides* and probably represents the first arbovirus isolation from diptera. This isolation was confirmed by successful serial passages and by serological tests. During the working period Tăhyňa virus was not handled in the laboratory. Furthermore antibodies to C/T38 were tested in the focus area in 600 domestic ducks, 52 cows and 8 sheep. Neutralizing antibodies in the titre  $\geq 16$  were found in 12 ducks (2%), 4 cows (7.7%), and 3 sheep.

Mosquitoes of genera *Aedes*, *Culex*, *Culiseta* and *Anopheles* are the best documented vectors of Tăhyňa virus (Bárdoš and Danielová, 1959; Bárdoš *et al.*, 1975a; Bárdoš *et al.*, 1975b; Lvov, 1972; Rosický and Málková, 1980). The interest of arbovirologists has focussed on the mass-occurring mosquitoes and the possible involvement of other vectors has been nearly neglected. Because Tăhyňa virus was several times isolated from the blood of patients (Bárdoš *et al.*, 1975; Sluka, 1972; Šimková, 1973; Lvov, 1977) and clinical infection has been documented by serological methods, its medical importance is evident. Therefore, the peculiarities of the natural foci of Tăhyňa virus should be further investigated by serological studies in up to now not monitored areas i. e. in those with an altitude 500 m or higher.

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