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SOME ECOLOGICAL ASPECTS ON TRIBEČ VIRUS *)

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Abstract. The incidence of virus-carrying ticks in various seasons ranged from 0.1 to 0.3 per cent in the Tribeč region. The virus strains identical with the prototype strain were isolated from *Clethrionomys glareolus* and *Pitymys subterraneus*. From the blood of sentinel pastured goats two virus strains were isolated, positive results were obtained from the partially engorged *I. ricinus* nymphs collected from the goat. Attempts to isolate Tribeč virus from the blood and milk of experimentally infected goats were unsuccessful. The results of our experiments suggest that Tribeč virus could circulate in nature between different instars of *I. ricinus* as vector and small mammals and domestic animals as hosts of this virus.

The Tribeč virus, belonging to the Kemerovo group of arboviruses was isolated in 1963 from *Ixodes ricinus* ticks in Tribeč mountain range, Slovakia (GREŠÍKOVÁ et al. 1965) and from *Haemaphysalis punctata* ticks in Roumania (TOPCIU et al. 1968). As summarized in Table 1, the estimated incidence of virus-carrying ticks

Table 1. Isolation of "Tribeč" virus from *Ixodes ricinus* ticks in 1963

Ixodes ricinus	Spring		Summer		Autumn	
	Number	Positive*) isolation experiments	Number	Positive isolation experiments	Number	Positive isolation experiments
Females	450	2/30	165	0/11	—	—
Males	420	2/16	150	1/5	18	0/1
Nymphs	1440	0/16	—	—	588	1/6
Engorged females	87	2/40	75	0/25	15	0/5
Engorged nymphs	147	1/15	85	0/8	79	0/8
	2544	7	475	1	700	1
Per cent		0.3		0.2		0.1

*) Number of virus strains isolated/number of pools examined.

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in various seasons ranged from 0.1 to 0.3 per cent. The incidence of infected ticks was the highest in the spring. Additional strains of Tribeč virus were isolated from *I. ricinus* ticks in 1965 and 1967 (Table 2). The highest incidence of virus-carrying ticks was found in the group of adults.

Table 2. Survey on Tribeč virus isolation from *I. ricinus* ticks during 1963—1968

Year	Locality	Number of isolation experiments									
		Total nymphs	Positive*)	Total ♀♀	Positive	Total ♂♂	Positive	Engorged nymphs	Positive	Engorged ♀♀	Positive
1963	Kostolany	2028	1/22	615	2/41	588	3/22	147	1/15	82	2/40
1964	Kostolany	477	0/5	248	0/16	208	0/7	—	—	—	—
1965	Kostolany	1967	0/21	391	0/17	395	0/20	—	—	—	—
1966	Kostolany	100	0/5	40	0/11	24	0/6	—	—	—	—
1967	Kostolany	508	1/16	187	0/17	170	1/13	—	—	—	—
	Žirany	272	0/27	163	1/33	156	0/31	—	—	—	—
	Topoľčianky	515	0/52	47	1/10	56	0/11	—	—	—	—
1968	Kostolany	1385	0/59	93	0/19	87	0/16	—	—	—	—
	Topoľčianky	1732	1/173	49	0/10	45	0/9	—	—	—	—

*) Number of virus strains isolated/number of pools examined.
— = not examined.

An effort was made to study the relationship of Tribeč virus to *I. ricinus* and *Dermacentor marginatus* ticks. Although serologically classified as a member of the Kemerovo group of arboviruses, its transmission by an arthropod vector under laboratory conditions has not yet been verified. Using the experimental feeding techniques, nymphs of *I. ricinus* ticks were fed on suckling mice infected with Tribeč virus. The experiments on the transstadial transmission of Tribeč virus were unsuccessful. We attempted, therefore, to obtain information about the relationship of Tribeč virus to tick tissues in an artificial way. We studied virus multiplication in intraperitoneally infected half-engorged female ticks and in tick tissue cultures. Half-engorged female ticks, 50—100 of either species, were inoculated intraperitoneally with $10^{6.5}$ mouse LD_{50} of Tribeč virus in 0.01 ml volumes. In *Dermacentor marginatus* females the virus was still found on the 43rd day after inoculation. From the 15th till the 43rd day it was irregularly detectable in titres of up to 10^3 mouse LD_{50} per 0.01 ml of the tick suspension (the experiment lasted from May 15 to June 12). In *I. ricinus* females, the virus persisted for 22 days

(from July 1 to 22) without deviations in the tirre levels except of a decrease between the 4th—11th day after infection (Fig. 1).

Using tick-tissue cultures, the multiplication of Tribeč virus was observed. The virus antigen could be demonstrated in the cytoplasm of tick-cell cultures by means of the fluorescent antibody techniques (ŘEHÁČEK et al. 1969). According to these results, *I. ricinus* ticks may be considered as suspected vectors of Tribeč virus.

Studying the natural vertebrate hosts, Tribeč virus was isolated in 1963 from the blood of live *Clethrionomys glareolus* and *Pitymys subterraneus*. The virus

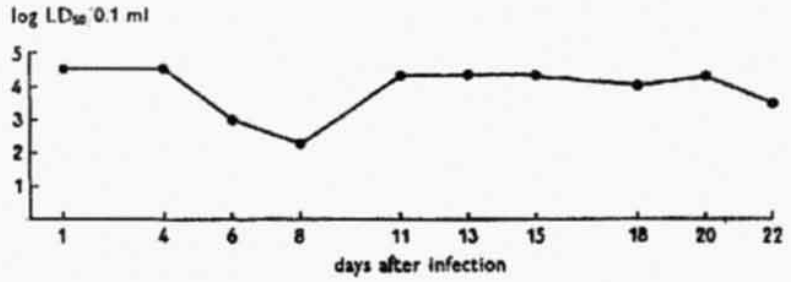

Fig. 1. Multiplication of Tribeč virus in *Ixodes ricinus* ticks (titrated in suckling mice).

isolation attempts from the blood of small rodents and insectivores, carried out from 1964 to 1968 yielded so far negative results. With reference to the contact of *C. glareolus* with *I. ricinus* ticks, these animals were inoculated subcutaneously with Tribeč virus. Viraemia was observed from the 2nd to the 7th day after virus inoculation. The titre of virus in the blood ranged from 10^2 to $10^{4.5}$ i.c. mouse LD_{50}, was found from the 2nd to the 3rd day after infection. The long lasting and intensive viraemia may suggest that *C. glareolus* may be a potential reservoir of Tribeč virus. On the other hand, two successive experiments on viraemia in experimentally infected *C. glareolus*, yielded negative results.

Studying a natural focus in Tribeč mountain range, fourteen goats as sentinel animals were pastured in the spring summer season in the locality of Horné Lefantovce. Two virus strains were isolated from the blood of a sentinel goat and one virus strain was isolated from 2 partially engorged *I. ricinus* nymphs collected from the goat. Neutralizing antibodies against Tribeč virus were found in the sera of two goats from which Tribeč virus strains were isolated on 14—79th days, eventually after virus isolation (ERNEK et al. 1966). Therefore four goats were inoculated subcutaneously with Tribeč virus mouse brain suspension. Blood samples for the demonstration of viraemia were taken at 24 hours intervals up to 10 days. Attempts to isolate Tribeč virus from the blood and milk of experimentally infected goats were negative. Additional experiments are needed to explain the role of domestic animals as hosts of Tribeč virus.

Finally, it is of interest that same localities in the Tribeč region are natural foci of tick-borne encephalitis virus (GREŠÍKOVÁ and NOSEK 1967), of Tribeč virus (GREŠÍKOVÁ et al. 1965) and of Uukuniemi virus (KOŽUCH et al. 1968). All above mentioned tick-borne arboviruses were isolated from *I. ricinus* ticks. It seems

necessary to pay attention to the persistence of these viruses in ticks in order to explain the possibility of double infection of ticks with arboviruses or interference between different tick-borne arboviruses.

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