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STUDIES ON THE ECOLOGY OF TICK-BORNE ENCEPHALITIS VIRUS IN THE CARPATHIAN AND PANNONIAN TYPES OF NATURAL FOCI

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Summary. — During the years 1972—1982, the role of *Ixodes ricinus* ticks was studied as vectors of tick-borne encephalitis (TBE) virus in the Carpathian and Pannonian types of natural foci. The proportion of TBE virus-infected *Ixodes ricinus* ticks in the Carpathian elementary foci varied from 0.37 to 4.1 per cent, while in the Pannonic elementary foci it ranged from 0.07 to 6 per cent, respectively. TBE virus was isolated from organs of small rodents in both the Carpathian and Pannonian types of natural foci. A correlation was established between the percentage of infected ticks and the proportion of seropositive inhabitants.

Key words: ecology; tick-borne encephalitis virus; Ixodes ricinus ticks; small rodents; types of the natural foci; surveillance

Introduction

In the Central Europe, *Ixodes ricinus* ticks show a characteristic curve of seasonal population with two peaks: the higher one occurring in April or May and the lower one in September and October (Černý, 1957; Pretzmann *et al.*, 1964; Nosek *et al.*, 1967; Radda *et al.*, 1967; Chmela, 1969; Nosek *et al.*, 1978). The natural foci of TBE occur in Slovakia in different ecosystems (Carpathian and Pannonic) which are more or less influenced by human activity. The stability of natural foci is determined by the trophic and topic relationships in the virus-vector-host chain (Nosek and Grulich, 1967; Nosek *et al.*, 1970, 1978; Blaškovič and Nosek, 1972; Radda, 1973). The original oak communities (xerophilic to mesophilic oak and mixed oak forest types) have been converted into pastures, meadows, cultivated steppe, forest-steppe, black locust forests and spruce forests. The original character of plant communities occurring in natural foci has been better preserved in the Carpathian type than in the Pannonian type of TBE natural focus.

We present the results of 10 years study on the role of *Ixodes ricinus* ticks in the ecology of TBE in these two types of TBE natural foci.

Materials and Methods

Virus isolation attempts. Virus isolations were made from ticks or from blood of live small rodents trapped in the natural foci. *Ixodes ricinus* ticks were collected from 1972 to 1984. The tick pools were prepared according to their stage and sex. The suspensions were made in Earle's

Table 1. Isolation of tick-borne encephalitis virus from Ixodes ricinus ticks in the natural foci of Slovakia

The type of natural focus	Year of collection	No. of examined ticks	No. of isolated strains	No. of pools/ No. of isolated strains	Locality	Month of collection	% of infected ticks
Carpathian	1972	24	1	1 F/1	Rača	June	4.1
		36	1	2 F/1	Jablonica	June	2.7
	1973	45	1	1 F/1	Bottovo	May	2.2
	1975	1874	1	50 N/1	Topolčianky	April	0.4
		34	1	1 M/1	Čierna dolina	April	2.9
	1978	135	1	10 N/1	Nemečky	May	0.7
	1979–1981	941	0	0	Považská Bystrica district	July	0.0
	1982	2373	4	10 N/1	Kurinec	April–May	0.8
				15 N/3		May	6.0
	1973	988	2	10 N/1	Devín	April	0.1
				5 F/1			1.1
Pannonian	1976	1420	1	5 F/1	Devín	April	0.07
	1978	1657	3	15 F/3	Devín	May	0.2
	1979	4481	2	20 N/2	Devín	April	0.33
	1980	1038	8	50 N/5	Gbelce	April-July	0.7
				5 F/1			

N = Nymphs F = Females M = Males

solution with 5% heated calf serum; they were spun at 3,000 rev/min, before inoculation (Grešíková and Nosek, 1967).

The suspensions from organs of small rodents were prepared in Earle's solution with 5% heated calf serum. After centrifugation as above, the samples were inoculated intracerebrally to suckling mice in 10 µl amounts.

The virus isolation technique, virus titration and identification of isolated strains had been described previously (Kožuch *et al.*, 1967; Grešíková and Nosek, 1982).

Serological examination. Human sera were examined by haemagglutination-inhibition (HI) tests (Clarke and Casals, 1958).

Results

A total of 16,183 *Ixodes ricinus* ticks were collected in Carpathian and Pannonic types of natural foci of TBE from 1972 to 1982. A high density of ticks was observed in areas with an increased concentration of small mammals and game (resting and feeding places), which were found in such zones as the border of forests, in clearings within intensively cultivated coniferous and deciduous forests, or along brooks. Very high densities of ticks occurred in forest used for grazing, or in rough pastures, and in forest-pasture areas with a high population of small rodents and insectivores (Table 1).

During the period of 10 years (1972—1982) altogether 26 strains of TBE virus were isolated from *Ixodes ricinus* ticks: 10 in the Carpathian type and 16 in the Pannonian type of natural focus of TBE. The proportion of infected ticks in both types of natural foci was 1.7 per cent. According to the development stages of *Ixodes ricinus* ticks, 6 virus strains were isolated from nymphs, 12 strains from the females and 8 strains from males. The proportion of infected *I. ricinus* ticks in the Carpathian elementary foci varied from 0.4

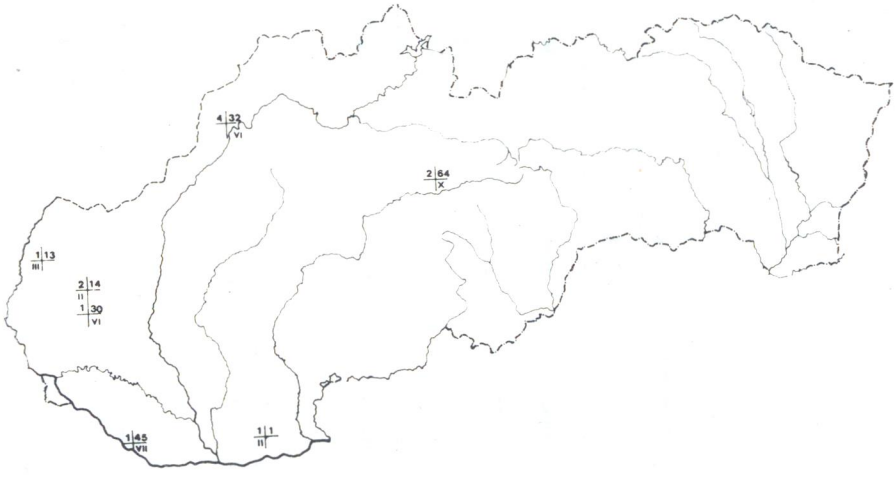

Fig. 1.

Isolation of the TBE virus strains from the organs of small rodents in Slovakia
Numerator: Number of isolated strains/Number of examined species;
Denominator: Month of collection

Table 2. Survey on TBE virus isolation from Ixodes ricinus ticks and HI antibodies to TBE virus in human healthy popuation

Type of natural focus	Locality	No. of examined ticks	No. of isolated strains	No. of pools/ No. of strains	Month of collection	Infected ticks (%)	Average	Antibodies in human sera (%)	Morbidity
Carpathian	Topolčianky	1565	5	40 N/4 5 F/1	July	0.31			
		1048	3	10 N/1 10 F/2	April	0.20	0.26	15—27	2.22
		1074	7	50 N/5 10 F/2	April	0.37			
Pannonian	Devín	2531	2	10 N/1 5 F/1	April	0.08			
		1420	1	5 F/1	April	0.07	0.11	7.4	0.02
		1657	3	15 F/3	May	0.18			

N = Nymphs; F = Females

to 4.1 per cent; in average 2.5% ticks have been found infected. The proportion of infected *I. ricinus* ticks in the Pannonian elementary foci varied from 0.07 to 6.0 per cent; in average 0.9 per cent of ticks were infected.

TBE virus strains were isolated from the organe of small rodents in the Carpathian as well as in the Pannonian types of natural foci. Virus isolation results from the organs of small rodents showed that TBE virus was detected from January till October (Fig. 1).

Examination of human healthy population by haemagglutination inhibition test for the presence of antibodies to TBE virus showed different percentage of seropositive inhabitants in the Carpathian type of natural focus as compared with the Pannonian type (Table 2). If the percentage of seropositive inhabitants was higher than 15, the natural focus was found to be active.

Discussion

Association of *I. ricinus* with different forest communities in the West Carpathians was demonstrated previously (Krippel and Nosek, 1978). The Carpathian type of TBE foci occurs in the southern and northern foreland of the Carpathians and is limited by the isotherme of average annual temperature of +8 °C. The Pannonian type of the natural focus of TBE occurs in lowland, especially in small forest enclaves occurring in highly cultivated landscape and is limited by the isotherms of average annual temperature from +8 °C to 11 °C.

The maintenance vector of TBE virus in either type of natural focus is the *Ixodes ricinus* tick, from which TBE virus isolation was reported in Slovakia as well as in the rest of Europe (Grešíková *et al.*, 1968; Hannoun, 1971; Nosek *et al.*, 1978; Kucheruk *et al.*, 1979; Grešíková and Nosek, 1982; Kožuch *et al.*, 1982). The infected ticks were distributed in "microfoci" (Pretzmann *et al.*, 1964; Nosek and Kožuch, 1970). The localities in which the virus was isolated from ticks or small vertebrates correspond to the isotherme of annual average temperature of +8 °C, with an average rainfall of 800 mm and with the isocoenoses of the first step of oak and with high population density of the chief vector (Nosek *et al.*, 1978). These isocoenoses with Pannonian type of climate (annual rainfall 550—700 mm, and annual average temperature of +8 °C to +11 °C) are characterized by relatively high population density of the *I. ricinus* tick (Nosek and Sixl, 1974).

The life cycle of *Ixodes ricinus* ticks last two or three years in the Central European lowlands but it may be extended up to more years at higher altitudes (Černý, 1957; Nosek *et al.*, 1967 Chmela, 1969; Černý *et al.*, 1974; Daniel *et al.*, 1976). Transmission of the TBE virus is influenced by the relationships between the vector and the hosts. Larger mammals and birds can disseminate the infected ticks for long distances. Small mammals are the maintenance hosts of TBE (Kožuch *et al.*, 1967; Grešíková and Nosek, 1982) as it has been demonstrated by TBE virus isolation from their organs in both the Carpathian (Kožuch *et al.*, 1967; Grešíková *et al.*, 1982) and the Pannonian type of natural focus (Kožuch *et al.*, 1982).

The surveillance on two types of natural foci of TBE: Carpathian and Pannonian was carried out not only by virus isolation attempts from ticks or from small rodents, but also by serological examination of human healthy population. The incidence of TBE virus in ticks corresponded to the antibody response in human healthy population and/or to the morbidity rate. Similar results were obtained in the Hercynic type of natural focus (Heinz *et al.*, 1979). A higher antibody response and higher morbidity rate was observed in the Carpathian than in the Pannonian natural focus. Finally, virus isolation from *Ixodes ricinus* ticks revealed that the TBE natural foci did not disappear in highly cultivated areas, e.g. TBE virus was isolated from *Ixodes ricinus* ticks in the locality Kurinec destined for recreation and tourism (Grešíková *et al.*, 1983).

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