MIXED NATURAL FOCUS OF TICK-BORNE ENCEPHALITIS, TULAREMIA AND HAEMORRHAGIC FEVER WITH RENAL SYNDROME IN WEST SLOVAKIA

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Summary. – Total of 923 small mammals of 7 species were collected in locality Záhorská Ves, West Slovakia, in 1990 – 1992. Among examined small mammal species it was *Clethrionomys glareolus* (48.7% of total, 17.5% positive for tick-borne encephalitis (TBE) virus antibodies), *Apodemus flavicollis* (29.7% of total, 17.5% positive), *A. sylvaticus* (11.3% of total, 16.3% positive), and *Microtus arvalis* (6.2% of total, 10.5% positive).The most abundant tick species (larval and nymphal stages) on small mammals was *Ixodes ricinus*. The extensity of infestation was 35.1 – 50.7%, and the intensity of infestation ranged in average from 4.1 to 7.8 ticks per animal. Out of 884 smalll rodent serum samples 16.9% had neutralizing antibody to TBE virus. Eight TBE virus isolates were recovered, six from *C. glareolus* and one each from *A. flavicollis and A. sylvaticus*; seven isolates were from brain tissue and one was from a pool of lung and liver tissues. One strain of *Francisella tularensis* was isolated from a pool of spleens of four *C. glareolus* collected in August 1991. Hantavirus antigens were detected in lung tissues of four *M. arvalis* collected in July and November 1990 – 1992. Antibody to Hantaan virus was detected by ELISA in one serum sample of *A. flavicollis* (titer 1:256) and antibody to Puumala virus in one serum sample of *C. glareolus* (titer 1:16).

Key words: tick-borne encephalitis; tularemia; haemorrhagic fever with renal syndrome; West Slovakia

Introduction

TBE, tularemia, and recently also haemorrhagic fever with renal syndrome (HFRS) are the most important zoonoses of rodents that are endemic in different geographic areas of Central Europe. High TBE virus and *F. tularensis* activities were recorded in last two decades in the Záhorie lowland, West Slovakia. TBE virus has been isolated from tissues of small rodents and neutralizing antibodies to this virus were detected in serum samples of birds, game, pastured cattle and rodents in the Záhorie lowland (Brummer-Korvenkontio *et al.*, 1980; Ernek *et al.*, 1975; Kožuch *et al.*, 1976; Kožuch *et al.*, 1983; Kožuch *et al.*, 1990). Similarly, *F. tularensis* has been isolated from small mammals and different tick species, mites and fleas in this region (Guryčová and Letkovský, 1973; Guryčová *et al.*, 1982).

Besides, antibodies to hantaviruses (Hantaan and Puumala serotypes) in serum samples of forest workers were found in this region (Kožuch, unpublished data).

In the study area of Záhorská Ves village several cases of TBE and tularemia, and one case of HFRS have been diagnosed and described in the past years (Krajčír, 1989; Guryčová, unpublished data; Grešíková *et al.*, 1994). The purpose of this study was to investigate the activity of the infections mentioned above and to find out whether they can be localized into a small area of two forest sites near the village Záhorská Ves.

Abbreviations: TBE = tick-borne encephalitis; HFRS = haemorrhagic fever with renal syndrome; CPE = cytopathic effect; ic = intracerebral; PS = porcine stable cell line; PBS = phosphate buffered saline

Materials and Methods

Description of the study area. Záhorská Ves village in Záhorie lowland is situated near Morava river. The sites of interest are located on the alluvial sands of the Morava river. This territory was formed up secondarily into 2 m high dunes. Pine and oak forests were the original stands here. Under the influence of man they have been changed into the present tree stands. In the part close to Záhorská Ves village Scotch pine (*Pinus sylvestris)* and British oak (*Quercus robur*) prevail. *Sambucus nigra, Ligustrum vulgare, Calamagrostis epigeios, Crataegus monogyna, Dactylis glomerata* and *Robinia pseudacacia* are mainly in the undergrowth. In the area of the forest path prevail on one side stands of false acacia (*Robinia pseudacacia*) and British oak (*Quercus robur*), while on the other site is a stand of alder (*Alnus glutinosa*). The undergrowth in the part of false acacia is poorer and is formed especially by synanthropic species of plants. The territory has a warm and moderately dry climate with moderate winters, mean annual temperature 9.6 °C and a total annual precipitation 610 mm.

Small mammals were live trapped in Swedish bridge metal traps using oat flakes as bait. Collection sites in the forest were about 1 – 3 km to the east from Záhorská Ves village. For the TBE studies, mammals were trapped once a month from June 1990 till December 1992, and for the investigation of *F. tularensis* from January 1991 till December 1992. The trap rate represented the average number of mammals trapped per 100 trap – nights. Insectivors were excluded from the examination because they were not live – trapped.

Infestation of trapped mammals by ticks was recorded. Tick species were identified, and the extensity and intensity of infestation were calculated.

Blood for serological examination was taken from *sinus orbitalis*. Sacrificed animals were necropsied and their organs (brains, livers, spleens and lungs) were taken aseptically.

TBE virus isolation. Homogenates of brains and livers were prepared in 2 ml of Eagle's Minimal Essential Medium containing 5% heat – inactivated newborn bovine serum and centrifugated at 3000 rpm for 15 mins. The supernatant fluids (0.01 ml) were used for ic inoculation of 1 – 4 day-old white mice. Five mice were used for each sample. Virus isolates were identified by neutralization test using 3 week-old white mice inoculated ic with mixtures of ten-fold dilutions of virus and undiluted hyperimmune mouse serum. The serum had neutralization index of 4.5 log units.

F. tularensis isolation. Spleen suspensions for isolation of *F. tularensis* were investigated in white mice by standard methods (Guryčová and Lysý, 1967; Guryčová *et al.*, 1982). The isolated strain was identified by a procedure of Guryčová (1980) and Guryčová *et al.* (1982).

Neutralizing antibodies to TBE virus in serum samples of rodents were examined with 100 $TCID_{50}$ of TBE virus harvested from cultures of porcine stable (PS) cell line. TBE virus exerts CPE on these cells (Kožuch and Mayer, 1975).

HFRS antigen was detected in 10% suspensions of lungs of small mammals (prepared in 1 ml PBS pH 7.2) by ELISA (Tkachenko *et al.*, 1981).

Hantavirus antibodies. Serum samples of collected rodents were tested for the presence of antibodies to hantavirus serotypes Hantaan and Puumala by indirect fluorescent antibody technique (Brummer-Korvenkontio *et al.*, 1980).

Results

A total of 923 small mammals of 7 species (*A. flavicollis, A. sylvaticus, C. glareolus, M. arvalis, S. araneus, S. minutus* and *C. leucodon*) were collected during 1990 – 1992. Out of 884 rodent serum samples 16.9% had neutralizing antibody to TBE virus.

In the trapped small mammals the frequency of individual species was as follows: *A. flavicollis* (29.7%), *A. sylvaticus* (11.3%), *C. glareolus* (48.7%), *M. arvalis* (6.2%), and *S. araneus* (3.5%). The prevalence of neutralizing antibody to TBE virus was 17.5 % in *A. flavicollis* (range 8.8 – 25.0 % in different years), 16.3% in *A. sylvaticus* (2.1 – 33.3%), 17.5% in *C. glareolus* (14.9 – 20.7%) and 10.5% in *M. arvalis* (0 – 25.0%) (Table 1).

The antibody prevalence in small rodents varied according to their sex and age, reaching in females 15.8% (11.6 – 20.4%

Table 1. Neutralizing antibody to TBE virus in small rodents in Záhorská Ves in 1990 – 1992

Species	Year of collection							
	1990 Positive/ tested	%	1991 Positive/ tested	%	1992 Positive/ tested	%	1990-1992 Positive/ tested	%
A.flavicolis	7/79	8.8	20/111	18.0	21/84	25.0	48/274	17.5
A.sylvaticus	1/47	2.1	7/30	23.3	9/27	33.0	17/104	16.3
C.glareolus	27/130	20.7	32/214	14.9	20/105	19.0	79/449	17.5
M.arvalis	0/25	0	4/24	16.6	2/8	25.0	6/57	10.5
Total	35/281	12.5	63/379	16.6	52/224	23.5	150/884	16.9

in different years) and in males 18.6% (14.0 – 27.5%). The antibody prevalence was 19.7% in adults (17.3 – 22.8%) and 14.4% in subadults and juveniles (7.3 – 23.5%) (Table 2). Titers of neutralizing antibody ranged from 1:4 to 1:16.

Table 2. Prevalence of neutralizing antibody to TBE virus in small rodents by sex and age in Záhorská Ves in 1990 – 1992

Species	Positive/tested (%)			
	Sex		Age	
	Females	Males	Adults	Subadults and juveniles
A.flavicolis	15.7	19.5	20.2	13.7
A.sylvaticus	15.0	17.6	13.9	18.0
C.glareolus	17.3	18.0	21.2	13.7
M.arvalis	15.8	18.6	7.1	11.6
Mean	15.8	18.6	19.7	14.4

Eight TBE virus isolates were recovered from organ tissues of rodents; six from *C. glareolus* and one each from *A. flavicollis* and *A. sylvaticus*; seven isolates were from the brain tissue and one was from a pool of lung and liver tissues. Four isolates were from small rodents collected in October, one each from animals collected in June, July, August and September (Table 3).

Table 3. TBE virus isolates from small rodents collected in Záhorská Ves in 1991 – 1992

Isolate No.	Species	Sex	Age	Month	Year	Tissue
1	*C.glareolus*	M	Adult	June	1990	liver,lung
92	*C.glareolus*	F	Adult	July	1990	brain
14	*A.flavicollis*	F	Subad.	Sept.	1990	brain
23	*C.glareolus*	F	Subad.	Oct.	1990	brain
209	*A.sylvaticus*	M	Subad.	Oct.	1990	brain
218	*C.glareolus*	F	Adult	Oct.	1990	brain
223	*C.glareolus*	F	Subad.	Oct.	1990	brain
612	*C.glareolus*	F	Adult	Oct.	1991	brain

M – male
F – female

Ixodes ricinus was the most abundant tick species (larvae and nymphs) on smmal mammals, while *Haemaphysalis concinna* was rare. The extensity of infestation was 35.1 – 50.7% and the intensity of infestation ranged in average from 4.1 to 7.8 ticks per animal. The trap rate was 26.2 – 49.6 (Table 4). The highest extensity and intensity of infestation were observed from May to August, and the highest antibody prevalence was found in May, July, and August; the highest trap rate was recorded from July to September (Fig. 1).

Table 4. Relationships between extensity and intensity of tick infestation, trap rate and TBE virus antibody prevalence in small mammals

Index	1990	1991	1992
Extensity[a]	50.7%	73.2%	35.1%
Intensity[b]	4.1	5.0	7.8
Trap rate[c]	49.6	40.7	26.2
Antibody prevalence	12.4%	16.6%	23.5%

[a]Percentage of animals infested with ticks.
[b]Average number of ticks per infested animal.
[c]Average number of animals trapped per 100 trap – nights.

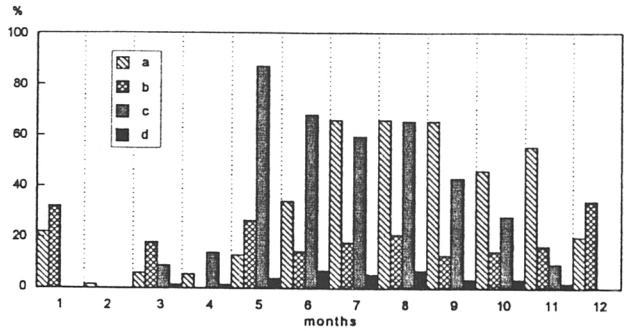

Fig. 1
Extensity and intensity of tick infestation, trap rate and TBE virus antibody prevalence in small mammals by months in 1990 – 1992
a – trap rate; b – antibody prevalence; c – extensity of infestation; d – intensity of infestation.

One strain of *F. tularensis* was isolated from a dominant rodent species *C. glareolus* collected in August 1991.

Hantavirus antigens were detected by ELISA in lung tissue of four *M. arvalis* collected in July 1990, November 1991 and November 1992. Antigen titers in lung suspensions were 1:32 – 1:64. No antigen was detected in lung tissue of the other small mammals. Antibody to Hantaan virus was detected in one serum sample of *A. flavicollis* (titer 1:256) and antibody to Puumala virus in one serum sample of *C. glareolus* (titer 1:16).

Discussion

Mixed natural foci of infection in West Slovakia have been described previously. Nosek *et al.* (1980) has described a mixed natural focus of TBE and lymphocytic choriomeningitis viruses together with *F. tularensis* and *Leptospira grippotyphosa* in Záhorie lowland. Similarly, a mixed natural focus of arboviruses (TBE and Uukuniemi) and *F.*

tularensis was observed in South-West Slovakia (Kožuch *et al.*, 1987).

In the present study of Záhorská Ves locality, the existence of mixed natural focus of TBE virus and hantaviruses together with *F. tularensis* was proved. In addition, neutralizing antibody to murine herpesvirus was found in 16.0% of serum samples of *A. flavicollis* in the same locality (Leššo J, unpublished data).

We have found the prevalence of neutralizing antibody to TBE virus in rodents of 16.9%. Differences were found according to the years: a rise of positivity from 12.5% (1990) up to 23.5% (1992) was observed. And *vice versa*, the number of TBE virus isolates was higher in 1990 (7 isolates) than in 1991 – 1992 (1 isolate).

The obtained results confirm that the most important hosts of TBE virus are the most abundant rodent species *A. flavicollis* and *C. glareolus*. This has been observed also in previous studies (Nosek *et al.*, 1982; Kožuch *et al.*, 1983; Kožuch *et al.*, 1990).The antibody prevalence in these animals was in correspondence with their infestation by larvae and nymphs of *I. ricinus* ticks.

M. arvalis represented only 6.2% of the examined small mammals. However, lung tissue of 4 from 57 examined *M. arvalis* were positive for hantavirus antigen (7.0%). This rodent species seems to be the most important host for hantaviruses in the study area. This is in contrast with the other areas in the East and South Slovakia, where hantavirus antigen was detected in the lungs of *A. flavicollis*, *A. sylvaticus* and *C. glareolus* (Grešíková *et al.*, 1986; Grešíková *et al.*, 1988).

The presence of *F. tularensis* in the focus can be important by causing the reduction of the rodent populations and subsequently affecting the circulation of the other pathogens in the focus.

The obtained results demonstrate a co-circulation of TBE virus, an unidentified hantavirus and *F. tularensis* in the study area.

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