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TICK-BORNE ENCEPHALITIS VIRUS ACTIVITY IN STYRIA, AUSTRIA

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Summary. - From 3 404 *Ixodes ricinus* ticks collected in 12 localities of Styria, Austria in 1990, 15 tick-borne encephalitis (TBE) virus isolates were recovered. Minimal field infection rate reached 4.4 virus containing ticks out of 1 000 collected ticks. Five isolates of TBE virus were obtained from target organs of *Apodemus flavicollis* trapped in locality Wagnitz. In a serosurvey based on virus neutralizing antibodies high prevalence of TBE virus was demonstrated in *A. flavicollis* (47.9 %) and *Clethrionomys glareolus* (29.4 %). These rodents formed 57.8 % and 41.0 % of 83 trapped small mammals.

Key words: tick-borne encephalitis; ticks; mammals; Austria

Introduction

Natural foci of TBE virus in Styria, Austria have been well documented since first isolations from *Ixodes ricinus* ticks (Kožuch *et al.*, 1973) and detection of virus neutralizing antibodies in small mammals (Sixl *et al.*, 1973) two decades ago. During the period of 20 years numerous human cases of viral meningoencephalitides were diagnosed as TBE virus infections in Styria (Radda 1978; Köck *et al.*, 1991; Stünzner *et al.*, 1991). All the published data show high TBE virus activity in natural foci of Styria.

The aim of the presented study was to collect recent field data on the tick infection rates and on the antibody prevalence in small mammals and to compare obtained results with those from previous studies in Styria and other adjacent territories.

Materials and Methods

The study area has been selected in submontaneous oak-hornbeam and oak-beech forests in the hilly country around Graz, Styria, Austria with mild and warm climate (Zimmerman *et al.*, 1989). Twelve localities have been selected south from Graz (Grambach, Fernitz, Keinach, Preding, Zwaring, Unterpremstätten), northeast from Graz (Wagnitz, Fasslberg, Not) and north from Gratz

(Kleinsemmering, Mortantsch, Kirchdorf). With the exception of Kirchdorf (800 m) all the other study sites were located at 400 to 500 m above sea level.

The questing ticks were collected by flagging the vegetation with a white woolen blanket. Ticks were kept at 4 °C in glass test tubes until further examination. Collected ticks were tested according to stage, sex and site of collection. Prior homogenization, ticks were washed in PBS supplemented with 200 IU of penicillin and 200 μg of streptomycin per ml. For homogenization, 10 unfed nymphs, 5 females or 5 males were pooled.

Small mammals were live-trapped into Swedish bridge metal traps and woody traps type "Chmela", using oat flakes as bait. The blood for serological examination was taken from the orbital sinus by the means of a pipette.

Humanly sacrified animals were necropsied and their organs (brain, lungs, liver and spleen) were aseptically collected. Tick and organ suspensions were prepared in Eagle's minimum essential medium (MEM) supplemented with 5 % heat-inactivated bovine serum and antibiotics and clarified by centrifugation at 3 000 rpm. Each suspension was inoculated intracranially (i.c.) into five 1 to 3 days-old albino mice, in a dose 0.01 ml per mouse. Serum samples were examined for the presence of neutralizing antibodies against 100 CPD_{50} of TBE virus on the cloned porcine stable (PS) cells in which this virus exerts a cytopathic effect (Kožuch and Mayer, 1975).

The viruses were identified in virus neutralization tests on suckling albino mice by i.c. inoculation of the mixture of virus and hyperimmune mouse serum against TBE virus (strain Hypr) with a neutralization index of 100 000.

Results

In total, 3 404 *Ixodes ricinus,* ticks were collected in 12 localities of Styria in May 14–18, 1990. From 2 445 nymphs 9 TBE virus isolates were recovered. From 451 female ticks 4 isolates, and from 508 male ticks 2 isolates have been obtained. Minimal field infection rate reached 4.4 virus containing ticks out of 1 000 collected ticks (Table 1). The titers of virus in a positive tick suspension varied from the lowest detectable value up to $10^{4.5}$ mouse i.c. LD_{50}/0.01 ml.

Five isolates of TBE virus were recovered and reisolated from the target organs of *Apodemus flavicollis* trapped in the locality Wagnitz, 4 from the mixtures of lungs, liver and spleen and one from a brain.

A serosurvey was performed based on virus neutralizing antibodies detected in small mammals collected in two localities (Grambach and Wagnitz) of Styria. A high prevalence of TBE virus was demonstrated in *Apodemus flavicollis* (47.9 %), the most abundant collected mammals species. The second abundant rodent species, *Clethrionomys glareolus* was also frequently in contact with TBE virus (29.4 %). Of 83 mammals tested (Table 2), 57.8 % were *A. flavicollis* and 41.0 % were *C. glareolus.*

Discussion

The obtained results demonstrated high activity of TBE virus in Styria, either by virus isolation, or as based on the serosurvey. The number of human cases in Styria (Radda, 1978) reflects these results from the study sites selected on the basis of reported human cases. In the natural foci of TBE virus (European

Table 1. TBE virus isolations from Ixodes ricinus ticks collected in Styria

Locality	Positive ticks/tested ticks				MFIR
	Nymphs	Females	Males	Total	
Grambach	1/501	0/84	1/117	2/702	2.9
Fernitz	1/10	0/1	0/5	1/16	62.5
Keinach	1/344	0/72	0/73	1/489	2.0
Preding	0/159	0/29	0/28	0/216	-
Zwaring	1/154	0/13	0/21	1/188	5.3
Unterpremstatten	0/9	0/27	0/19	0/55	-
Wagnitz	0/50	0/15	0/31	0/96	-
Fasslberg	0/245	0/12	0/11	0/268	-
Not	0/136	0/10	0/12	0/158	-
Kleinsemmering	3/412	1/106	0/106	4/624	6.4
Mortantsch	2/310	2/45	1/55	5/410	12.2
Kirchdorf	0/115	1/37	0/30	1/182	5.5
Total	9/2445	4/451	2/508	15/3404	4.4

MFIR - minimal field infection rate; calculated ratio of positive ticks to 1000 tested ticks

Table 2. TBE virus neutralizing antibodies in small mammals collected in two localities of Styria

Locality	Grambach		Wagnitz	
Species	Animals positive/tested	% positive	Animals positive/tested	% positive
Clethrionomys glareolus	1/4	25.0 %	9/30	30.0 %
Apodemus flavicollis	6/16	37.5 %	17/32	51.3 %
Crocidura leucodon	-	-	0/1	0 %
In total	7/20	35.0 %	26/63	41.3 %

subtype) in other geographic areas of Central Europe the minimal infection rate is usually much lower (Radda *et al.*, 1969; Molnár *et al.*, 1978; Kožuch *et al.*, 1987). Also the antibody percentage was usually lower, e.g. 14.6 % in western Slovakia (Kožuch *et al.*, 1990), 15.8 % in northern Moravia (Kožuch *et al.*, 1976) and 13.3 % in northern Austria (Kožuch *et al.*, 1969).

The principal role of *A. flavicollis* mice as the maintenance hosts of TBE virus was pointed out not only by the high occurence of antibodies (47.9 %), but also by the virus isolations from the target organs. At present, the role of the persistent infection in small mammals for the maintenance of TBE virus in nature is unclear. Further study in highly active natural foci of TBE virus similar to those in Styria in comparison to the foci in other European territories would be of great interest.

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