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EXPERIMENTAL CHARACTERISTICS OF VIRAEMIA CAUSED BY TWO STRAINS OF TICK-BORNE ENCEPHALITIS VIRUS IN SMALL RODENTS

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Summary. — Two strains of tick-borne encephalitis (TBE) virus differing in virulence for subcutaneously (s.c.) inoculated adult white mice elicited different levels of viraemia in small rodents. Strain Skalica of a lowered virulence caused no detectable viraemia in adult *Clethrionomys glareolus* and only negligible viraemia in adult *Apodemus sylvaticus*, *Microtus arvalis* and *Pitymys subterraneus* species. The virulent strain 204 gave rise to higher levels of viraemia, sufficient for infection of TBE virus vectors-ixodid ticks, in most individuals of the rodent species tested. Viraemia surpassing the threshold values of infectivity for tick vectors was also found in some juvenile and adult *Clethrionomys rufocanus*, *C. rutilus* and *Micromys minutus*. The level of viraemia depended on rodent species and age, and exhibited individual variability.

Key words: tick-borne encephalitis virus; *small rodents*; *viraemia*; *ecological markers*

Introduction

Investigation of viraemia in adult *Clethrionomys glareolus* infected s.c. with 12 strains of TBE virus collected in different geographical areas and different types of natural foci revealed that 9 strains of the virus elicited viraemia surpassing the threshold values of its infectivity for ixodid ticks (2.5—4.5 log LD_{50}/0.03 ml), but the remaining 3 virus strains caused only lower levels of viraemia (0.4—1.5 log LD_{50}/0.03 ml) (Čunichin *et al.*, 1978). The ability or inability of various TBE virus strains to cause in adult *C. glareolus* such levels of viraemia which surpass the threshold level of virus infectivity of its vectors — ixodid ticks was considered to represent an ecological marker of TBE virus (Chunikhin and Kurenkov, 1979).

The present investigation deals with the level of viraemia in 8 species of small rodents after inoculation with 2 strains of TBE virus possessing different virulence after experimental s.c. infection.

Materials and Methods

Experimental animals. Adults of the following species were used: *Clethrionomys glareo us C. rufocanus, C. rutilus, Microtus arvalis, Pitymys subterraneus, Apodemus flavicollis, Apodemus sylvaticus and Micromys minutus.* All adult rodents were trapped in nature. *M. arvalis* also subadult and juvenile individuals were used; these always came from one family and F_1 generation, and were kindly supplied by the Institute for Vertebrate Zoology, Czechoslovak Academy of Sciences, Brno.

Virus strains. Two TBE virus strains with different virulence for s.c. inoculated adult white mice were employed: the virulent strain 204 (Grešíková, 1975), and strain Skalica of a lowered virulence (Grešíková and Sekeyová, 1980). Both virus strains were propagated in suckling mouse brains (SMB) after intracerebral (i.c.) inoculation. Strain 204, isolated from *Ixodes ricinus* females collected in the vicinity of Bratislava, was in its 10 th SMB passage; strain Skalica, isolated from the brain of *C. glareolus* trapped in West Slovakia (Grešíková et al., 1976), was in its 4 th SMB passage.

Inoculation of animals and identification of recovered virus strains. All animals were inoculated s.c. with brain suspensions containing 10^4-10^5 SM i.c. LD_{50}/0.1 ml of virus. To demonstrate viraemia, blood was taken from the orbital sinus into 1 % heparin solution on days 2—3 post infection (p.i.) (assumed peak of viraemia). Blood samples were titrated in 1—3 days old suckling white mice inoculated i.c. with 0.03 ml volumes and virus titres were expressed in log LD_{50}/0.03 ml values. Viruses recovered from the blood were identified in virus neutralization (VN) tests in adult white mice inoculated i.c. with a mixture of virus and hyperimmune goat serum whose neutralization index was 10^4.

Table 1. Viraemia and HI antibodies in Clethrionomys species inoculated with two strains of TBE virus

Virus strain Skalica				Virus strain 204			
Animal No.	Viraemia* on day 2	Viraemia* on day 3	HI titre day 14	Animal No.	Viraemia* on day 2	Viraemia* on day 3	HI titre day 14
Clethrionomys glareolus							
1	0	0	20	6	<1	2.0	20
2	0	0	20	7	2.2	1.7	20
3	0	0	10	8	1.9	2.0	40
4	0	0	10	9	3.0	1.5	40
5	0	0	20				
Clethrionomys rufocanus							
1	1.2	2.7	10	6	2.5	2.5	40
2	1.2	2.0	10	7	3.0	1.8	80
3	0	1.0	0	8	1.5	2.5	20
4	0	1.0	0	9	3.2	1.0	80
5	2.0	2.0	20	10	1.5	2.8	40
Clethrionomys rutilus							
1	2.0	1.5	0	6	2.7	1.5	80
2	2.5	<1	10	7	3.0	2.2	80
3	1.0	1.0	10	8	1.8	3.5	40
4	0	1.5	10	9	3.2	—	20
5	0	1.0	10				

* Virus titres in the blood (log i. c. mouse LD_{50}/0.03 ml); 0 means no virus detected.
Either strain was inoculated in doses of 4—5 log LD_{50}/0.1 ml. — = Not tested.

Serological tests. Before virus infection the small rodents were bled and pre-tested for the presence of neutralizing antibodies to TBE virus on PS cells as described (Kožuch and Mayer, 1975). The haemagglutination inhibiting (HI) antibodies were assayed according to Clarke and Casals (1958) by both the macromethod and micromethod on plastic plates on day 14 p.i. Sera used in the HI test were treated by acetone. Antigens were prepared by sucrose-acetone extraction.

Results

Rodent sera tested before virus inoculation contained no neutralizing antibody to TBE virus. Rodents inoculated s.c. with a dose of 10^4-10^5 LD_{50}/0.1 ml of either strain of TBE virus showed no manifest clinical signs of disease, with an exception of *Pitymys subterraneus* adults which died on days 11–13 p.i. with strain 204. The virus titres in 10 % brain suspensions from dead animals reached 6.7 mouse i.c. LD_{50}/0.03 ml.

Virus titres (log LD_{50}/0.03 ml) in the blood of the small rodents studied varied at the peak of viraemia (days 2–3 p.i.). Strain Skalica caused no viraemia in *C. glareolus*; in *C. rufocanus* and *C. rutilus* viraemia was either absent or present in low titres (1.0–2.7). All individuals of the 3 *Clethrionomys* species tested developed viraemia in titres from 1.0–3.5 after infection with strain 204 (Table 1).

Table 2. Viraemia and HI antibodies in Microtus arvalis of different age inoculated with two strains of TBE virus

Virus strain Skalica				Virus strain 204			
Animal No.	Viraemia on day 2	Viraemia on day 3	HI titres day 14	Animal No.	Viraemia on day 2	Viraemia on day 3	HI titres day 14
Adults							
1	0	< 1	0	31	< 1	1.5	20
2	0	0	0	32	1.0	2.0	20
3	0	0	0	33	4.1	2.0	20
4	2.0	0	0				
Subadults							
14	2.0	< 1	0	10	3.0	4.0	40
15	< 1	2.1	0	8	3.3	2.0	–
16	< 1	0	0	13	2.0	3.0	20
17	0	0	0	12	3.5	2.8	20
18	2.2	< 1	0	11	3.6	2.8	40
19	2.0	2.3	0				
20	< 1	0	0				
21	2.5	< 1	0				
22	2.1	< 1	0				
Juveniles							
1	< 1	3.3	20*	11	3.0	2.5	–
2	1.5	< 1	20*	12	2.6	2.2	1280*
3	< 1	1.0	20*	13	3.0	3.5	1280*
4	2.0	< 1	20*	14	3.4	1.0	320*
5	1.0	< 1	40*	15	2.6	2.4	–

*Macromethod of HI test.
< 1 = Traces of virus.
For other details see Table 1.

Viraemia was not detected in the majority of *M. arvalis* adults infected with strain Skalica; if present, it reached only low titres (<1.0—2.0). Subadult and juvenile *M. arvalis* exhibited viraemia in titres from <1.0—2.5 and <1.0—3.3, respectively. By contrast, higher titres of viraemia from <1.0—4.1 were observed in *M. arvalis* of all age groups after infection with strain 204 (Table 2).

Infection of *P. subterraneus* with strain Skalica resulted in a very low titre (1.0) of viraemia, whereas strain 204 caused viraemia reaching very high titres (up to 6.7). In *M. minutus* infected with strain Skalica, viraemia was found regularly, except one case, in titres from 1.0—2.7, and after infection with strain 204 all animals developed viraemia in titres from 1.3—3.3 (Table 3).

Similar results were obtained with *Apodemus* species (Table 4), i.e. absence or low levels of viraemia in both *A. flavicollis* and *A. sylvaticus* after infection with strain Skalica, but higher levels of viraemia after infection with strain 204, reaching titres of 5.2 and 3.2 in *A. flavicollis* and *A. sylvaticus*, respectively.

The titres of HI antibodies in the blood of the rodents at 14 days p.i. varied depending on the strain of virus and the method of HI test used. After infection with strain Skalica the HI antibody titres were very low

Table 3. Viraemia and HI antibodies in Micromys minutus and Pitymys subterraneus inoculated with two strains of TBE virus

Virus strain Skalica				Virus strain 204			
Animal No.	Viraemia on day 2	Viraemia on day 3	HI titres day 14	Animal No.	Viraemia on day 2	Viraemia on day 3	HI titres day 14
Micromys minutus							
1	1.0	2.0	—	11	2.6	2.5	640
2	< 1	1.5	—	12	1.3	2.0	640
3	2.5	1.0	80	13	1.6	2.4	—
4	2.7	< 1	80	14	2.2	3.3	2560
5	2.0	< 1	—	15	2.0	1.5	2560
6	1.0	0	—				
7	1.6	2.0	—				
Pitymys subterraneus							
1	< 1	< 1	< 40	11	1.6	3.5	320
2	1.0	1.0	< 40	12	2.0	4.0	+
3	0	< 1	< 40	13	4.0	6.0	+
4	< 1	0	80	14	5.0	6.7	+
5	1.0	< 1	< 40	15	1.5	5.0	+
6	< 1	< 1	40	16	3.2	3.8	640
7	1.0	< 1	40	17	4.3	6.2	+

* The macromethod of HI test was used. + = *P. subterraneus* Nos 12, 13, 14, 15 and 17 died before day 14.

For other details see Table 2.

Table 4. Viraemia and HI antibodies in Apodemus species inoculated with two strains of TBE virus

	Virus strain Skalica				Virus strain 204		
Animal No.	Viraemia on day 2	Viraemia on day 3	HI titres day 14	Animal No.	Viraemia on day 2	Viraemia on day 3	HI titres day 14
Apodemus flavicollis							
2	< 1	0	20	6	< 1	1.0	20
3	0	0	20	7	2.5	2.5	20
4	< 1	< 1	10	8	2.6	3.0	20
5	< 1	0	10	9	1.6	1.0	20
				10	5.2	2.0	40
Apodemus sylvaticus							
1	1.5	< 1	10	6	3.2	2.5	80
2	2.0	< 1	10	7	2.0	2.8	40
3	2.0	< 1	10	8	2.8	2.5	40
4	< 1	< 1	0	9	2.5	> 1	40
5	1.5	< 1	0	10	1.5	3.0	40

For explanations see Table 2.

(10—20) or absent by the micromethod, but higher (20—80) by the macromethod. Similarly, in the blood of rodents infected with strain 204, the HI antibody titres varied from 20—80 by the micromethod, but reached higher values (320—2560) by the macromethod (Tables 1—4).

Discussion

After s.c. inoculation of adults *Clethrionomys glareolus*, *C. rutilus*, *Microtus arvalis*, *Pitymys subterraneus*, *Apodemus flavicollis*, *A. sylvaticus*, *Micromys minutus*, as well as of juvenile and subadult *M. arvalis* with the virulent strain 204 of TBE virus, we observed higher levels of viraemia than the threshold level of infectivity for ixodid ticks (Radda *et al.*, 1969). On the other hand, infection of small rodents with strain Skalica possessing lowered virulence for white mice after s.c. inoculation (Grešíková and Sekeyová, 1980) resulted in negligible or undetectable viraemia. However, strain Skalica exceptionally caused viraemia surpassing the threshold levels of infectivity for tick vectors in some juvenile and subadult individuals of *M. arvalis*, and in adult *C. rufocanus*, *C. rutilus* and *Micromys minutus*.

All species of small terrestrial mammals so far studied are known as hosts of TBE virus in natural foci (Mornsteinová and Albrecht, 1957; Ernek *et al.*, 1963; Málková *et al.*, 1965; Kožuch *et al.*, 1967; Kucheruk *et al.*, 1969; Radda *et al.*, 1969). The differences observed in experimental pathogenicity of TBE virus strains can be explained by inhomogeneity of the animals used (individual variability, different age), species specificity, and variability of the investigated virus strains.

In our study both the development of viraemia and reaching of its threshold levels of infectivity for virus vectors varied depending on rodent genus, species, and age, as well as on the virus strain used. Differences were also

found in the height of antibody response the levels of HI antibodies were much higher after inoculation with the virulent strain 204 than with the low virulent Skalica in all rodent species tested.

Maintenance in nature of TBE virus strains with decreased peripheral virulence for white mice could be realised by some species of small rodents (or their juvenile individuals) and insectivora which may serve as hosts of such virus variants. Moreover, the role of some species of ixodid ticks as virus vectors cannot be excluded either. The problem of circulation in nature of TBE virus strains with lowered virulence remains to be elucidated by further studies.

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