

MARKER STABILITY OF THE SKALICA STRAIN (FROM THE TICK-BORNE ENCEPHALITIS COMPLEX) PROPAGATED IN *IXODES RICINUS* TICKS

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Summary. — *Ixodes ricinus* larvae from the laboratory breed were infected on viraemic suckling mice inoculated with the Skalica strain from the tick-borne encephalitis complex. The strain recovered from individual nymphs on days 39 and 54 after metamorphosis had the markers (ic⁺, sc⁻, t⁻, v⁻) identical with the original Skalica strain.

Key words: Skalica strain (tick-borne encephalitis complex); propagation in ticks; markers of virulence

During the field studies in Western Slovakia the Skalica strain belonging to the tick-borne encephalitis (TBE) complex was isolated from *Clethrionomys glareolus* (Grešíková *et al.*, 1976). The Skalica strain was found to be thermostabile (t⁻), non-pathogenic for white mice following the subcutaneous inoculation (sc⁻) causing only a threshold viraemia of short duration in adult outbred mice (v⁻) (Grešíková and Sekeyová, 1980; Rajčáni and Grešíková, 1982).

It was of interest to investigate whether the Skalica strain would retain its stability after passage in *Ixodes (I.) ricinus* ticks.

Ten suckling mice were infected intracerebrally (i.c.) and subcutaneously (s.c.) with the Skalica strain (7.5 log LD₅₀/0.01 ml). *I. ricinus* larvae from the laboratory breed, 200 in number, were infected by sucking on viraemic newborn mice. The virus titres in individual larvae ranged between 2—2.5 log LD₅₀/0.01 ml. On the days 39 (sample No. 1) and 54 after metamorphosis, individual nymphs were examined. The virus titres were determined in suckling mice as previously described (Grešíková and Nosek, 1967); they reached in nymphs similar values (2—2.5 log LD₅₀/0.01 ml) as in the larvae.

The reisolated virus and the stock strain were examined for thermostability (t marker), for pathogenicity in adult white mice after s.c. inoculation (sc marker) and its ability to induce viraemia in these mice (v marker). The methods employed were described previously (Grešíková and Sekeyová, 1980).

The growth of virus strains passaged in the ticks (samples No 1 and No 3) was investigated in chick embryo cells at 37 °C and 41 °C. The titre of the Skalica strain isolated after metamorphosis of ticks decreased at 41 °C by 3.1 to 3.5 log units, whereas the titre of the prototype TBE virus strain did not change substantially (Table 1).

Table 1. Thermosensitivity of the Skalica strain after passages in *Ixodes ricinus* ticks

	The titre of inocula (log mouse LD ₅₀ /0.01 ml)	Extracellular virus titre (log mouse LD ₅₀ /0.01 ml) in CEC cultures after 18 hr incubation at	
		37 °C	41 °C
Skalica (sample No. 1)	7.5	4.5	1
Skalica (sample No. 3)	8.5	4.7	1.6
Prototype TBE virus strain (Hypr)	8.3	7.0	6.6

The pathogenicity for adult white mice of the strains reisolated from the nymphs was unchanged. Thus, the Skalica strains previously propagated in *I. ricinus* ticks were found non-pathogenic for adult white mice after s.c. inoculation (marker sc⁻). In the same mice viraemia was not found on the 2nd day after virus inoculation, and only traces of the virus (1 log LD₅₀/0.01 ml) were detected on day 3 (marker v⁻). The Skalica strain did not change its sc⁻ property even after the second metamorphosis of ticks (from nymphs to adults). Females of *I. ricinus* ticks were fed on adult white mice and the virus was again isolated from the ticks, but the feeder mice did not become ill.

The marker stability of the Skalica strain during the interstadial development of *I. ricinus* ticks was verified. The virus strains recovered from nymphs were ic⁺, sc⁻, t⁻ and v⁻, i.e. they showed properties identical with the original Skalica strain. The TBE virus strains isolated from *I. ricinus* ticks in natural foci displayed marked differences in the ic and sc characters; they kept the ic⁺ and sc⁺ properties distinct from strains in higher mouse passages; 93% of the former were sc⁺ and only 6.8% were sc[±] (Mayer and Kožuch, 1969). On the other hand, the selected clone E₅"14" of the original E₅ Langat strain showed the ic⁺ sc⁻ t⁻ character (Mayer and Mitrová, 1977).

Analysing the data reported in the literature (Price *et al.*, 1963; Pogodina and Savinov, 1964; Mayer and Kožuch, 1969; Mayer and Mitrová, 1977) and recently obtained results, it appears that the Skalica strain preserves its ic⁺ sc⁻ t⁻ v⁻ character after passaging in *I. ricinus* ticks. In addition, the successful transmission of Skalica strain to white mice by viruliferous *I. ricinus* nymphs indicates the possibility of virus circulation in natural foci.

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