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SHORT COMMUNICATIONS

REPLICATION OF TICK-BORNE ENCEPHALITIS (TBE) VIRUS IN IXODES RICINUS TICKS

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Abstract. The influence of external factors on virus carriage of *Ixodes ricinus* ticks in laboratory and in nature was studied. In laboratory experiment, only one nymph was positive for the presence of virus on 120th day after metamorphosis. The virus titer was 10^2 mouse i.c. LD_{50}/0.03 ml. Transmission experiments were negative. The nymphs were positive on 75th, 111th and 159th day after metamorphosis, always after chilling in the field experiment. The titres of virus varied from the lowest detectable amount value to $10^{3.6}$ mouse i.c. LD_{50}/0.03 ml. The transmission of virus was positive in two cases.

This paper is a continuation of our studies on the replication of tick-borne encephalitis (TBE) virus in *Dermacentor marginatus* ticks (Nosek and Kožuch 1985). The effect of diapause of *I. ricinus* ticks on multiplication of the TBE virus in them has been studied by Mishaeva and Erofeeva (1979). These authors stated that the TBE virus multiplied more intensively in ticks developing without diapause in laboratory conditions. The aim of our study was to compare the influence of external factors on virus carriage of ticks in laboratory and under natural conditions.

MATERIAL AND METHODS

Infection of ticks. *I. ricinus* ticks coming from the laboratory—bred F_1 generation were used. The larvae of *I. ricinus* were infected by feeding on viraemic suckling white mice on August 17, 1981. These mice were infected with 10 per cent brain suspension of newly isolated strain of the TBE virus in 4th mouse passage. Titer of 10 per cent brain suspension reached 10^8 i.c. mouse LD_{50}/0.03 ml. The feeding of larvae lasted 2—3 days. The engorged larvae were divided into two groups each numbering 200 larvae. The larvae were kept in a glass cylinder and placed in an alder-pine forest (September 5, 1981) in a typical locality of its occurrence. The ticks in laboratory experiment were kept at a temperature of 20—22 °C and 90 % RH. **Virological examination.** The suspensions were prepared from individual engorged larvae, engorged nymphs and unfed nymphs in 1 or 0.5 ml of basal Eagle solution with 10 per cent of inactivated calf serum and inoculated i.c. to suckling mice with a dose of 0.01 ml. The positive samples were titrated on the white mice weighing 8—10 g i.c. with a dose of 0.03 ml. Chilling of ticks, lasting 4 days at +4 °C, was used. Before the experiment with the transmission of virus, a feeding capsule was attached to each mice and 24 hours later individually infected nymphs were placed in each capsule.

RESULTS

Laboratory experiment. The titer in engorged larvae varied from 10^1—10^3 mouse i.c. LD_{50}/0.03 ml. The premoulting period (larva-nymph) lasted 25 days. Nymphs, always 10 in number, were individually examined 52, 120, 156, 164, 204 and 239 days after metamorphosis. On 120th day after metamorphosis and chilling, one nymph was positive for the presence of virus (titer 10^2 mouse i.c. LD_{50}/0.03 ml). The

other isolation experiments were negative. Transmission experiments, each with 10 nymphs used (120 and 204 days after metamorphosis), were negative. The virophorous period (larva-nymph) lasted 148 days.

Field experiment. The premoulting period (larva-nymph) lasted 72 days. Nymphs, always 10 in number, were individually examined for the presence of virus on the 11th, 75th, 111th, 119th, 159th, 194th and 209th day after metamorphosis. The nymphs were positive on the 75th, 111th and 159th day after metamorphosis. The titers of virus varied from threshold value to $10^{3.6}$ mouse i.c. LD_{50}/0.03 ml, always after chilling (Table 1). The transmission experiment (111th day after metamorphosis) was positive in two cases. The virus titers in fed nymphs varied from threshold value to $10^{3.8}$ mouse i.c. LD_{50}/0.03 ml. The ticks examined on the 11th day after metamorphosis as well as on 119th day after metamorphosis without previous chilling were negative. Negative results were also obtained in ticks examined on 194th day after metamorphosis and previous chilling. The virophorous period lasted up to 234 days.

Table 1. Virus carriage in *Ixodes ricinus* ticks infected with TBE virus in field experiment

Ticks No	Day after metamorphosis	Virophorous period	Virus in ticks (log i.c.mouse LD_{50}/0.03 ml)
1—10	11	86	0
11	75	150	3.6
12—13	75	150	0
14	75	150	< 1
15—16	75	150	0
17	75	150	2.5
18—19	75	150	0
20	75	150	1.6
21	111	186	3.0
22	111	186	< 1
23—30	111	186	0
31—40	119	194	0
41	159	234	1.5
42	159	234	1.6
43—49	159	234	0
50	159	234	< 1
51—60	194	269	0
61—70	209	284	0

DISCUSSION

The field experiments with larvae and nymphs of *I. ricinus* tick resulted in a longer metamorphosis, increased vitality of virus carriage, and transmission of the TBE virus in comparison with laboratory experiments. The fluctuating temperature in nature as well as solar radiation prolonged the metamorphosis. The influence of external factors on the physiological state of ticks and virus replication seems to be evident. By means of short chilling the activity of ticks began and also transmission of virus was possible. Conversely, in ticks kept at non-fluctuating laboratory temperature, the survival of virus as well as the life span of ticks was much shorter than in field conditions. We have observed that the ageing of ticks in laboratory

conditions passed more rapidly than in nature and survival of virus was then shorter. The ageing process in nature passes more slowly as confirmed by our field experiment. (Nosek and Kožuch 1985). Despite the chilling of the ticks 269—284 days old the absence of virus was observed; it was probably due to the decreasing metabolism of the tick host. It is evident that the results of laboratory experiments cannot be interpreted under natural conditions.

РЕПЛИКАЦИЯ ВИРУСА КЛЕЩЕВОГО ЭНЦЕФАЛИТА У КЛЕЩЕЙ *IXODES RICINUS*

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Резюме. Изучали влияние диапаузы на вирусоносительство клещей вида *Ixodes ricinus* в лабораторных и природных условиях. В лабораторном опыте только одна нимфа оказалась позитивной на присутствие вируса 120 дней после метаморфоза. Титр вируса был 10^2 мышиных интрацеребральных LD_{50}/0,03 мл. Опыты по передаче вируса дали отрицательные результаты. Нимфы реагировали положительно 75, 111 и 159 дней после метаморфоза, всегда после охлаждения в полевом опыте. Титры вируса варьировали между самым низким поддающимся обнаружению количеством и $10^{3.6}$ мышиных интрацеребральных LD_{50}/0,03 мл. В двух случаях передача вируса осуществилась.

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