SURVIVAL OF TBE VIRUS DURING THE LIFE OF DERMACENTOR RETICULATUS NYMPHS

The aim of our study was to demonstrate the survival and level of TBE virus in moulting ticks during ecliptic phase, prefeeding period up to death of nymphs. The species *D. reticulatus* (Fabr.) is a known vector of this virus.

Dermacentor reticulatus larvae (400 in number) originated from our laboratory colonies and were infected by feeding on viraemic suckling mice. The suckling mice were infected intraperitoneally with 0.1 ml of 10 % brain suspension of TBE virus hedgehog strain J 13 from the 9th mouse passage. The virus in the 10 % suspension was 10^8 mouse intracerebral LD_{50}/0.03 ml. Viraemia in white mice ranged the values from $10^{4.8}$ to $10^{5.5}$ mouse ic LD_{50}/0.03 ml. The larvae were infected at May 19, 1975. The engorged larvae dropped off on 3rd day after attachment. After feeding they were immediately tested for the presence of virus and 100 % were confirmed as viruliferous. The suspensions from engorged larvae and unfed nymphs were prepared individually in 1 ml of basic Earle's solution with 10 % inactivated calf serum. (Fig. 1). Isolations of virus from individual suspensions were carried out on 1—3 day-old suckling mice and the positive suspensions were titrated intracerebrally in white mice weighing 6—8 g with 0.01 ml or 0.03 ml respectively. The average titers were calculated each from 3 individuals.

The virus titer in engorged larvae showed a slow decrease on 2nd day and an evident increase on 5th day after engorgement. The discs in moulting nymphs were observed on 8th day, and the hatching started on 12th day. During this period the virus titer reached the threshold level (Fig. 1). On 16th day the level of virus titre in unfed nymphs reached the value of 1.6 $\log_{10}$ ic mouse LD_{50}/0.03 ml, with maximum 2.5 $\log_{10}$ ic mouse LD_{50}/0.03 ml on 19th day. A very clear decrease of virus titer was observed on 30th day when the titer repeatedly reached the threshold value. The second phase of increasing titer in unfed nymphs was observed on 37th day, and it lasted to 46th day in titer ranging from 2.5 to 2.2 $\log_{10}$ ic mouse LD_{50}/0.03 ml. The nymphs spontaneously died on 47th day.

The small decrease of virus titer in engorged larvae agrees with the findings (A. Radda, Annual Report Institute of Hygiene, University Vienna from 1965—1966) in *Ixodes ricinus* larvae. The fluctuation of titers in unfed nymphs may be in correlation with metabolic changes during the life of nymphs and show approximately two weeks'cycles. The mentioned fluctuation seems to be in correlation with transmission experiments and is a phenomenon occurring as well in vectors of animal viruses as in vectors of plant viruses. Similar fluctuation was also observed in experiments with the leafhopper *Macrosteles fascifrons* transmitting the aster yellows agent (I. Kunkel, Amer. J. Botany 13: 646—705, 1926). The moulting period as well as the ecliptic phase in *D. reticulatus* nymphs is very short similarly as in other short-living ticks. It may be concluded that the nymphs were viruliferous throughout their life-time.

O. KOŽUCH and J. NOSEK
Institute of Virology,
Slovak Academy of Sciences,
Bratislava, ČSSR

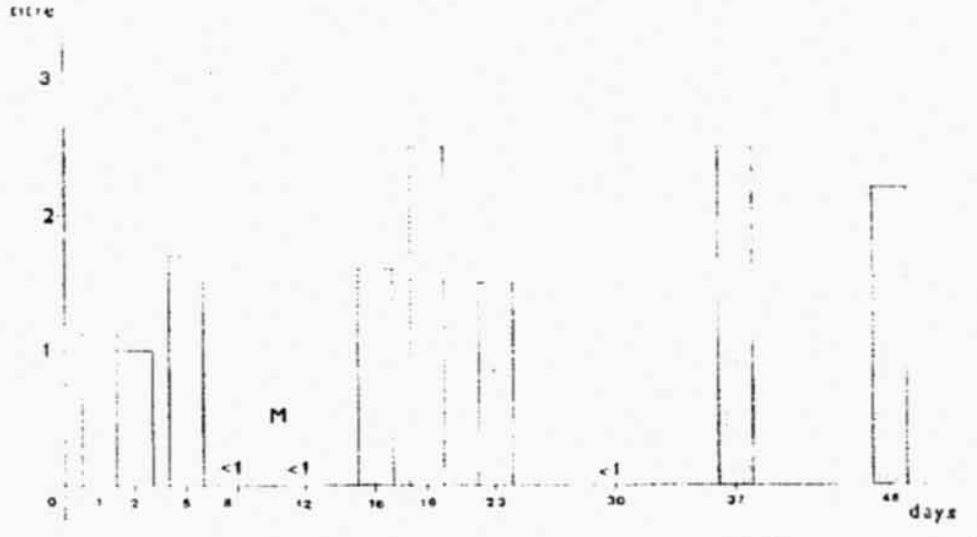

Fig. 1. The titre of virus in engorged larvae and unfed nymphs of *Dermacentor reticulatus* tick. M — moulting period, titre is given per 0.03 ml.