

The survival of the Tick-Borne Encephalitis (TBE) virus in nymphs of *Haemaphysalis inermis* tick and its transmission to pygmy mouse (*Micromys minutus*)

J. NOSEK
O. KOŽUCH
J. LYSÝ*

Résumé

MAINTIEN DU VIRUS DE L'ENCÉPHALITE A TIQUES (TBE) CHEZ LES NYMPHES D'HAEMAPHYSALIS INERMIS ET TRANSMISSION A LA SOURIS NAINNE MICROMYS MINUTUS

Le virus de l'Encéphalite à Tiques (TBE) (souche 204) se maintient chez les nymphes d'*Haemaphysalis inermis* durant 37 à 60 jours. Ce virus a été transmis avec succès par les nymphes de cette espèce à six souris naines, *Micromys minutus*, dans le temps record minimum de repas sanguin de 90 minutes. Les titres des anticorps neutralisants dans les sérums de souris ont atteint la valeur de 1/8.

Les auteurs étudient ensuite les relations entre les vecteurs (*H. inermis*), dont les nymphes piquent volontiers l'homme, et l'hôte vertébré (*Micromys minutus*) dans les foyers naturels d'encéphalite à tiques.

Mots-clés : Ixodidae – Nymphes – Transmission – Rongeurs – Encéphalite – Région paléarctique.

Summary

The tick-borne encephalitis virus, strain 204, survived in *Haemaphysalis inermis* nymphs for 37 or 60 days respectively. This virus was successfully transmitted with *H. inermis* viruliferous nymphs in minimal feeding time ($T_{im} = 90$ minutes) to six pygmy mice (*Micromys minutus*). The titres of N-antibodies in sera of all pygmy mice reached the value 1 : 8.

The coincidence of the TBE virus, *H. inermis* nymphs and *Micromys minutus* was discussed.

Key words : Ticks – Nymphs – Transmission – Rodents – Encephalitis – Palearctic region

INTRODUCTION

Isolation of TBE virus from adults of *H. inermis* tick has been recorded by Grešíková and Nosek (1966). The virus was also transmitted from nymphs of

to white mice. Pathogenicity of the TBE virus for pygmy mouse was recorded by Mornsteinová and Albrecht (1957); Kožuch *et al.* (in press).

MATERIAL AND METHODS

The *H. inermis* viruliferous nymphs were originated from laboratory bred. The ticks were kept at

viraemic suckling white mice. They fed about one hour. The mice were inoculated i.p. by the TBE virus strain 204 (Grešíková, 1975) with 10 % mouse brain

* Institute of Virology, Institute of Experimental Biology and Ecology, Slovak Academy of Sciences, Bratislava, Tchécoslovaquie.

suspension of titre 10^8 i.c. mouse/0.01 ml. The all engorged larvae were viruliferous. The virus titre in engorged larvae ranged from 10^4 - 10^8 i.c. mouse LD_{50} per 0.01 ml. The moulting period lasts 35 days, the prefeeding period 37 or 60 days.

The transmission of the TBE virus to pygmy mouse was carried out by feeding of viruliferous nymphs of *H. inermis* on fixed or on nonfixed mice each kept in Erlenmayer flasks during feeding respectively. The feeding of nymphs lasts 1.5-3 hours. The mice used in experiment originated from nature. Before the experiment, the blood was taken from sinus orbitalis for serological examination. All individuals were negative for N-antibodies against TBE virus.

The pygmy mice 6 in number and 18 viruliferous nymphs were used in transmission experiments. The blood for presence of N-antibodies was taken at 14th day after infection and examined on PS cells (Kožuch and Mayer, 1975).

The transmission experiments were carried out in autumn period (the second half of September and beginning of October). The engorged nymphs were examined individually for presence of virus. The suspensions were prepared in 1 ml of basal Eagle medium (BEM) with 10 % of inactivated calf serum. The suspension were titrated i.c. on 1-3 days old white suckling mice with a dosis of 0.01 ml.

RESULTS

The viroforic period in *H. inermis* nymphs lasted 72 or 95 days respectively. The all pygmy mice used in experiments were negative for presence of N-antibodies against TBE virus. The positive transmission of the TBE virus by bite of viruliferous *H. inermis* nymphs was confirmed by presence of N-antibodies against the TBE virus at 14th day after the tick bite. The titers of N-antibodies in sera of all pygmy mice reached the value of 1 : 8. The titre of individually examined nymphs ranged from $10^{3.5}$ - $10^{3.8}$ mouse i.c. LD_{50} /0.01 ml. It was established that also the feeding time of 90 minutes was sufficient for infection of pygmy mice.

DISCUSSION

The coincidence of *Micromys minutus* with the TBE virus in natural foci of tick-borne encephalitis virus may come in consideration. *Micromys minutus*

is a frequent member in small mammalian synusia, especially in lowland. The TBE virus was successfully transmitted in laboratory experiments to young roe deer (*Capreolus capreolus*) and to common shrew (*Sorex araneus*) (Nosek *et al.*, 1967 ; Kožuch *et al.*, 1967)

The high titre of virus in *H. inermis* nymphs and relatively short feeding showed that nymphs have different physiology. The very short transmission time (Tt) lasting 90 minutes is stressed and it is at present minimal time of transmission.

Haemaphysalis inermis tick requires a very high degree of humidity because it occurs on the place with mild winters, Mediterranean and coast of Black Sea and on the Continent in river basins. In northern area of its distribution the seasonal incidence of adults belongs to the humid season (October-May) where it is bounded to the very warm forest steppe localities (on andesite or andesite pyroclasts) with mild winter. Season incidence of larvae and nymphs belongs prevalently to the dry season but their feeding is very short. Larvae are active from the end of May to the end of August (beginning of September). Nymphs appear from the middle April to the end of October. Larvae and nymphs are most active at morning after retreat of dew and before evening. In nymphs the normal activity ranges between 10° - 22° C by 100 % rel. humidity. The nymphs infest frequently humans. The reaction after their bite is immediate and strong.

Manuscrit reçu au Service des Éditions de l'O.R.S.T.O.M.
le 7 janvier 1981.

REFERENCES

- GREŠKOVÁ (M.), 1975. — Isolation of Bratislava strains of tick borne encephalitis virus from *Ixodes ricinus* ticks in Devín natural focus. *Brat. lek. Listy*, 64 : 52-57 (in Slovak).
- GREŠKOVÁ (M.) and NOSEK (J.), 1966. — Isolation of tick-borne encephalitis virus from *Haemaphysalis inermis* ticks. *Acta virol.*, 10 : 359-361.
- KOŽUCH (O.) and MAYER (V.), 1975. — Pig kidney epithelial (PS) cells : a perfect tool for study of flaviviruses and some other arboviruses. *Acta virol.*, 19 : 498.
- KOŽUCH (O.), NOSEK (J.), LICHARD (M.), CHMELA (J.) and ERNEK (E.), 1967. — Transmission of tick-borne encephalitis virus by nymphs of *Ixodes ricinus* and *Haemaphysalis inermis* to the common shrew (*Sorex araneus*). *Acta virol.*, 11 : 256-259.
- KOŽUCH (O.), ČUNICHIN (S. P.), GREŠKOVÁ (M.), NOSEK (J.), KURENKOV (V. B.) and LYSÝ (J.). — The ecological marker « Clg » of the TBE virus in different rodent-species. *Acta virol.* (in press).

TRANSMISSION OF TBE VIRUS FROM *HAEMAPHYSALIS INERMIS* TO PYGMY MOUSE

- MORNSTEINOVÁ (D.) and ALBRECHT (P.), 1957. — Experimental infection of *Micromys minutus* with Czechoslovak tick-borne encephalitis virus. *Čs. Epidem. Mikrobiol. Immunol.*, 4 : 157-161 (in Czech).
- NOSEK (J.), KOŽUCH (O.), ERNEK (E.) and LICHARD (M.), 1967. — Übertragung des Zeckenencephalitis Virus nach Zeckenbiss der virophorischen Nymphen von *Haemaphysalis inermis* und Weibchen von *Ixodes ricinus* auf die Rehkitzten (*Capreolus capreolus*). *Zbl. Bakt. I. Orig.*, 203 : 162-166.
- NOSEK (J.), SIXL (W.) and WALTINGER (H.), 1973. — Morphology of *Haemaphysalis (Alloceraea) inermis* Bir. tick in relation to its behaviour and ecology. 1. Intern. Arbeitskolloquium über Naturherde von Infektionen in Zentral-Europa, 17-19 April 1973, Illmitz und Graz, p. 130-138.
- SIXL (W.) and NOSEK (J.), 1971. — Einfluss von Temperatur und Feuchtigkeit auf das Verhalten von *Ixodes ricinus*, *Dermacentor marginatus* und *Haemaphysalis inermis* Zecken. *Arch. Sci. Genève*, 24 : 97-100.