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ISOLATION OF TBE VIRUS FROM THE TICK IXODES HEXAGONUS

K. KŘIVANEC, J. KOPECKÝ, E. TOMKOVÁ and L. GRUBHOFFER

Institute of Parasitology, Czechoslovak Academy of Sciences, České Budějovice

Abstract. Tick-borne encephalitis (TBE) virus was isolated from a sample of *Ixodes hexagonus* ticks collected from the hair of the western European hedgehog (*Erinaceus europaeus*) which had been captured on the edge of a new housing estate in České Budějovice at the end of September 1986. This was the first isolation of TBE virus from this vector, supporting the previous experimental results. The virus was identified in immunofluorescent and plaquereduction neutralization tests.

Under the conditions of Central Europe the main vector of tick-borne encephalitis is the three-host tick *Ixodes ricinus*. TBE virus was moreover isolated from another three representatives of the family Ixodidae. Until recently TBE virus has not been isolated from *I. hexagonus* ticks, only Streissle (1960) conducted successful experimental infection with it. While *I. ricinus* ticks play an unequivocal role in the transmission of TBE virus to man, the role of other ticks in the maintenance of this virus in natural foci is still not clear.

MATERIALS AND METHODS

A total of 120 adult *I. hexagonus* ticks and 100 nymphs of this species were collected from the hair of the western European hedgehog (*Erinaceus europaeus* L.) which had been captured on the edge of a new housing estate in České Budějovice (old field lane leading to the woods) on 26 September 1986. The adults were separated into four groups with 30 specimens each, the fifth group consisting of nymphs. From these samples homogenized suspensions were prepared in phosphate buffer (pH 7.4), containing 3% bovine fetal serum, 200 U/ml penicillin and 200 μg/ml streptomycin.

The suspensions were clarified by centrifugation and inoculated intracerebrally with a dose of 0.01 ml and subcutaneously with 0.02 ml into one-day-old white mice. The brains of the dead suckling mice were homogenized in 10% suspension in PBS with antibiotics and bovine fetal serum and following clarification this suspension was then used to infect white mice of different age and PS cells, on which the virus titration and neutralization test by plaque method after De Madrid and Porterfield (1969) were carried out.

The PS cells in the L-15 medium with 3% bovine serum were infected on Linbro panels (24 wells) and then cultivated under overlay with 0.75% of carboxymethylcellulose. The panels were stained with naphthalene red in acetic acid solution 4—5 days p.i. The plaque-reduction neutralization test proper was conducted after incubation of consecutive tenfold dilutions of the isolate with constant dilution of the mouse hyperimmune anti-TBE serum (titre in NT 1 : 256) and of the normal control serum 1 : 10 for the period of one hour at 37 °C.

From the brain suspensions of dead suckling mice clarified 10% suspensions were used for virus titration on both the suckling and adult white mice; these brains were also used for the preparation of saccharose-acetone antigen (Clarke and Casals 1958) in order to find out the hemagglutination activity of the isolate. Impression films of the infected brains were fixed for 10 minutes with cooled acetone and stained with mouse hyperimmune anti-TBE serum and conjugate SwaM/FITC (Sevac Praha) diluted 1 : 10 in PBS.

RESULTS

Five sterile suspensions from adult ticks and nymphs of *I. hexagonus* were bacteriologically tested and from the sample 166-2 (30 adults) a viral strain was isolated which was identified in plaque-reduction neutralization test as tick-borne encephalitis virus. The strain 166-2 was neutralized by a specific anti-TBE serum (NI = 3.1 log). The re-isolation test from the original suspension was also successful. Likewise the result of immunofluorescent test confirmed that it was indeed TBE virus.

The newly isolated strain of TBE virus was multiplied on mouse brains and showed the titre 6×10^7 PFU/ml. In the second i.c. passage on suckling white mice the virus showed the titre $10^{6.3}$ $SMicLD_{50}$/ml, while in subcutaneously inoculated adult white mice the titre ranged about 10^5 LD_{50}/ml. The saccharose-acetone antigen repeatedly prepared from the brains of suckling white mice lacked hemagglutination activity and was therefore inapplicable in HI test.

DISCUSSION

Many isolation proofs on the presence of TBE virus in its main vector, the tick *I. ricinus* (Hubálek 1986) were recorded from the territory of Czechoslovakia. This virus was also demonstrated in other representatives of the family Ixodidae, the ticks *D. marginatus* (Libíková and Albrecht 1959) and *H. inermis* (Grešíková and Nosek 1966). Two strains of TBE virus isolated from the tick *H. concinna* (Riedl et al. 1971) were reported from Austria. Streissle (1960) conducted extensive tests with TBE virus in *I. hexagonus* ticks, in which he demonstrated transstadial transmission of the virus and its multiplication in this secondary vector. Subsequent successful experiments were carried out with *D. reticulatus* (Nosek et al. 1984) and *H. concinna* (Kožuch and Nosek 1980), in which the virus also multiplies and is transmissible. Moreover, the long-lasting maintenance of TBE virus was also experimentally demonstrated in the species *I. arboricola* (Lichard and Kožuch 1967) and *H. spinigera* and *H. turturis* (Nosek et al. 1967).

Overlooked should not be the communication of Nosek et al. (1982) who expressed a suspicion that in natural foci of montane type in the Low Tatras in Czechoslovakia the circulation of TBE virus is primarily maintained by the *I. trianguliceps* ticks; due to this fact previous isolations of TBE virus from small terrestrial mammals in the High Tatras are explained from sites free from *I. ricinus* ticks (Bárdoš et al. 1959).

While the tick *I. ricinus* transmits TBE virus to a great number of hosts of different ecological vertebrate groups, the burrow tick *I. hexagonus* is the vector parasitizing under normal conditions primarily hedgehogs, small carnivores (Mustelidae) and foxes (*V. vulpes*). Consequently it can transmit viral infections only among a relatively narrow spectrum of hosts.

The significance of the hedgehog in the propagation of TBE virus was pointed out by Rosický (1953) who assessed in detail the vectors in natural foci by demarcating in the process of transmission a separate hedgehog zone which holds good primarily in the vertical spread of tick-borne encephalitis. However, the role of hedgehogs in the propagation of this disease has not been elucidated enough to this day. While detecting antibodies to TBE hedgehogs *E. roumanicus* in the Tribeč area Kožuch et al. (1967) found 17 % to be positive and in two cases (out of 53 animals) isolated directly TBE virus from their blood. Kožuch and Nosek (1964) called attention to an interesting possibility of alimentary infection of the hedgehog with TBE. Hedgehogs *E. roumanicus* were fed on white mice infected with the strain Hypr and viremia in hedgehogs was demonstrated between day 2 and 10; at the same time neutralization antibodies to TBE were also found in all laboratory animals. The above authors also drew attention to the significant role of post-hibernation viremia in hedgehogs in further circulation of TBE virus, this fact having been earlier pointed out by Tongeren (1958).

The TBE strain isolated by us can be appraised as pathogenic virus isolate which is of interest due to its vector (*I. hexagonus*) because the virus has not been so far isolated from it in free nature. The western European hedgehog from whose hair the ticks were collected, may be an important factor in the formation of new TBE natural foci even while migrating in the suburban and urban highly cultivated landscape, where the main TBE vector, the tick *I. ricinus* also occurs in high numbers.

At present altogether five tick species are listed as TBE vectors confirmed by isolation. Moreover, at least five other tick species are suspected of their participation in the circulation of TBE virus in natural foci. Despite the fact that in the mechanism of transmission of TBE virus to man solely the species *I. ricinus* is incriminated, the role of other secondary vectors in the circulation and maintenance of this medically important infection should be further elucidated.

ВЫДЕЛЕНИЕ ВИРУСА КЛЕЩЕВОГО ЭНЦЕФАЛИТА (КЭ) ИЗ КЛЕЩА *IXODES HEXAGONUS*

К. Крживанец, Я. Конецкий, Е. Томкова и Л. Грубгоффер

Резюме. Вирус клещевого энцефалита (КЭ) выделили из выборки клещей вида *I. hexagonus*, собранных из шерсти европейского ежа (*Erinaceus europaeus*), которого поймали на окраине микрорайона в Чешских Будейовицах в конце сентября 1986 г. Данный случай является первым выделением вируса КЭ из этого переносчика, подтвердившим таким образом прежние экспериментальные результаты. Идентификацию вируса проводили с помощью реакции иммунофлуоресценции и реакции нейтрализации по редукции бляшек.

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K. K., Parazitologický ústav ČSAV,
Branišovská 31,
370 05 České Budějovice, ČSSR

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C. Jura: Bezkregowce. Zarys morfologii, systematiki, filogenezy. (Invertebrates. Review of morphology, systematics, phylogeny.) ***Państwove Wydawnictwo Naukowe, Warszaw 1986, 602 pp. 224 Figs. Price 660 Zl.***

In 1986 a new textbook of the zoology of invertebrates appeared in the People's Republic of Poland. The textbook is voluminous, containing 571 pages of text, an extensive index (10 pages) and 224 drawings. It is also completed by very nice black-and-white photographs (66 altogether). It is devoted to the representatives of the subkingdom Metazoa. In the introduction the author gives a short outline of information from general zoology (embryonal development structure of tissues and organs). He also describes the symmetry and origin of invertebrate animals. The main contents of the textbook are the characteristics of the individual animal phyla. In the system of the subkingdom Metazoa altogether 24 phyla are ranked. The characterization of the phyla follows a uniform pattern. First there is a description of the general properties of the phylum followed by the description of the external structure of the body, internal structure of the body, reproduction and evolution. Then follows a systematic list of classes and orders and a detailed description of individual classes — external and internal structures, body cover, nervous system, sense organs, digestive system, respiratory organs, excretory and genital systems, way of reproduction and evolution. For each phylum a systematic outline of classes and orders, examples of the most interesting representatives and views of the phylogenetic origin are given.

Twenty-four animal phyla are described in detail in the textbook: 1. Mesozoa, 2. Spongiaria, 3. Cnidaria, 4. Ctenophora, 5. Platyhelminthes, 6. Nemertini, 7. Aschelminthes, 8. Kamptozoa, 9. Annelida, 10. Priapulida, 11. Sipunculida, 12. Echiurida, 13. Arthropoda, 14. Onychophora, 15. Linguatulida, 16. Tardigrada, 17. Mollusca, 18. Tentaculata, 19. Echinodermata, 20. Chaetognatha, 21. Pogonophora, 22. Protochordata, 23. Tunicata, 24. Acrania.

The author introduces as the first one the phylum Mesozoa, unlike the conception of the zoological system in the textbooks of other authors (Shulc and Gvozdev 1970, Dogel 1981, Grabda E. 1984) where these animals are ranked as a class of the phylum Platyhelminthes. Contrary to a general concept of the zoological system of many authors (such as Remane 1978, Gaisler 1983, Hickman et al. 1986, etc.) Tunicata and Acrania are ranked here as independent phyla. Chordata are characterized briefly as a group with an independent phylum Vertebrata which, however, is no longer described in the textbook.

Views concerning the evolution of invertebrates are given in the conclusion. The animal kingdom is divided into two subkingdoms — Protozoa and Metazoa. The starting group for Metazoa are Protozoa.

Despite this interesting and original conception of the zoological system it is possible to evaluate the textbook very positively. It is written in a brief style and clearly arranged, thus enabling the students of biology to acquire a good knowledge of the zoology of invertebrates. It is recommended as a textbook for university students of biology.

Dr. L. Vojtková, D.Sc.