BIOLOGY OF TICK-BORNE ENCEPHALITIS VIRUS

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Summary. – Tick-borne encephalitis (TBE) virus is an important human pathogen belonging to the genus *Flavivirus* within the family *Flaviviridae*. The genome of the TBE virus is a single-stranded RNA (ssRNA) molecule of positive polarity encoding all the viral proteins within a single open reading frame (ORF). TBE virus shares common physical and genetic characteristic of the flavivirus genus. Two subtypes of the TBE virus have been described: (1) European, endemic in many parts of Europe and transmitted by *Ixodes ricinus* ticks, and (2) Far Eastern (Russian spring summer encephalitis (RSSE) virus), endemic in Far East and transmitted by *Ixodes persulcatus* ticks.

Key words: tick-borne encephalitis virus; biology

Introduction

TBE virus is a member of the TBE virus group of the genus *Flavivirus* of the family *Flaviviridae* (Westaway *et al.*, 1985; Murphy *et al.*, 1995). The taxonomy of the genus *Flavivirus* is based on cross-neutralization tests with polyclonal hyperimmune mouse ascitic fluids prepared against each of the viruses. Nine serogically defined groups are recognized within this genus (Calisher *et al.*, 1989; Murphy *et al.*, 1995). One of them is the TBE virus group which includes TBE virus, Karshi virus, Kyasanur Forest disease virus, Langat virus, louping ill virus, Omsk haemorrhagic fever virus, Powassan virus, Royal Farm virus, Carey Island virus, and Phnom-Penh bat virus.

Abbreviations: aa = amino acid; CF = complement fixation; CNS = central nervous system; CPE = cytopathic effect; ELISA = enzyme-linked immunosorbent assay; HI = haemagglutination-inhibition; i.c. = intracerebral; i.s. = intraspinal; nt = nucleotide; MoAb = monoclonal antibody; ORF = open reading frame; p.i. = post infection; RSSE = Russian spring-summer encephalitis; RT-PCR = reverse transcriptase-polymerase chain reaction; ssRNA = single-stranded RNA; TBE = tick-borne encephalitis; VN = virus neutralization

TBE virus has two subtypes:

(a) European subtype (Hanzalova virus, Hypr virus, Kumlinge virus, Neudoerfl virus), and

(b) Far Eastern subtype (RSSE virus, Absettarov virus and Sofyn virus (Fig. 1).

The antigenic relationships among the viruses of the TBE virus group were studied by monoclonal antibodies (MoAbs) (Grešíková and Sekeyová, 1984, 1990). According to these studies the viruses Powassan, Karshi and Royal Farm seem to differ from others of the TBE virus group.

On the other hand, the Far Eastern and European subtypes of TBE virus are closely related antigenically. They are distinguishable on the basis of antibody absorption, haemagglutination-inhibition (HI) and agar diffusion tests (Clarke, 1962; Rubin and Chumakov, 1980). This antigenic distinction has been confirmed at the molecular level by use of MoAbs (Heinz *et al.*, 1982) and by nucleotide sequencing (Mandl *et al.*, 1988; Pletnev *et al.*, 1990). These subtypes can be distinguished also on the basis of peptide maps (Heinz and Kunz, 1982) and amino acid sequences. They share homologies of their structural proteins of 86 – 96 % (Mandl *et al.*, 1988; Yamshchikov and Pletnev, 1988) and differ in their tick vector: the European subtype is transmitted by *I. ricinus* ticks, and the Far Eastern subtype by *I. persulcatus* ticks (Table 1).

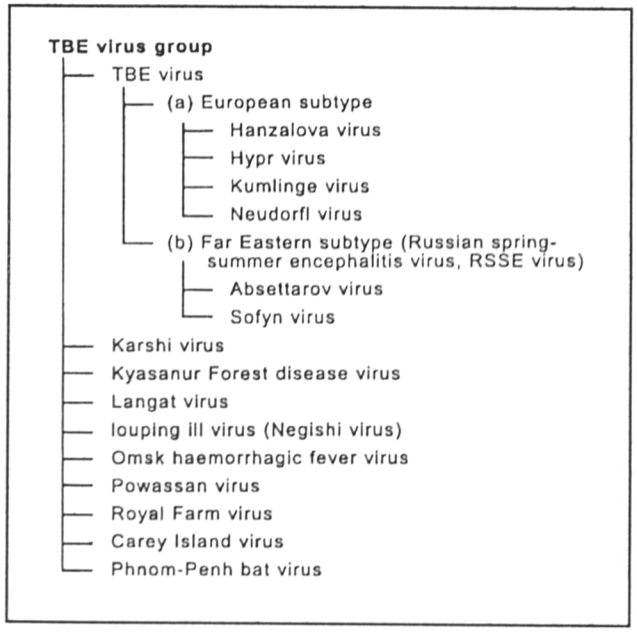

Fig. 1
TBE virus group

Table 1. Differentiation between European and Far Eastern subtypes of TBE virus

TBE virus	European subtype	Far Eastern subtype
Genome coding capacity	3414 aa long polyprotein	3412 aa long polyprotein
Main vector	*Ixodes ricinus*	*Ixodes persulcatus*
Severity of disease	Relatively mild	Severe
Disease synonyms	Central European TBE, Czech TBE, Slovak TBE, biphasic meningo--encephalitis, TBE	Far Eastern TBE, Far East Russian encephalitis, Taiga encephalitis, TBE
Case fatality rate	1-5%	8-54%
Distribution	Europe (except Benelux and Portugal)	Far Eastern Russia (Primorie, Khabarovsk, Krasnojarsk, Altai, Tomsk, Omsk, Kemerovo, Ural, Priural and Western Siberia)

Remarkable stability of the genome and antigenic determinants of TBE virus have been demonstrated in TBE viral strains isolated from different biotops in Europe (Guirakhoo *et al.*, 1987). Hovewer, most of TBE virus strains have been adapted to the mouse brain.

Spherical virions of flaviviruses (diameter of 40 – 50 μm) consist of a core (diameter 30 μm) and an envelope with 6 μm long surface projections. The core contains genome, a linear single-stranded RNA of positive polarity (10,488 nt long in TBE virus) and RNA-associated core protein C. The flavivirus genome contains a single long ORF encoding a polyprotein which consists of 3414 aa in Neudoerfl virus (Mandl *et al.*, 1988, 1989; Heinz and Mandl, 1993) and 3412 aa in Sofyn virus (Pletnev *et al.*, 1990). The polyprotein is posttranslationally cleaved with viral protease (protein NS3). The gene order in the genomic RNA is 5'-C-M-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3'. Membrane protein M and glycoprotein E are components of the envelope and spikes. The envelope contains also lipids and saccharides derived form the host cell. Protein NS5 is a part of the RNA-dependent RNA polymerase, protein NS3 is helicase and glycoprotein NS1 is released from infected cells as a soluble complement-fixing antigen. The envelope glycoprotein E has receptor binding and fusion activities and induces neutralizing and protective antibodies (Heinz *et al.*, 1981a).

It is known that a single aa substitution in glycoprotein E at aa 384 (Tyr by His) leads to a strongly reduced patogenicity of the virus after its peripheral inoculation into adult mice (Holzmann *et al.*, 1990). On the other hand, serial passages of a TBE virus, isolated from organs of a bank vole in Slovakia, through the salivary glands of *I. ricinus* ticks lead to a reduction of virulence of the virus for laboratory mice. The attenuated virus has two aa substitutions in E protein, one which makes it different from both parental and Neudoerfl viruses (Glu to Lys at aa 84) and another which makes it identical to Neudoerfl virus (Ile to Thr at aa 319) (Labuda *et al.*, 1994). But after consecutive mouse brain passages of the attenuated mutant, the original virulent phenotype appeared again, however, the two aa substitutions remained conserved (Kaluzová *et al.*, 1994).

TBE virus grows in cultures of Detroit-6, Hela, PS, SPEV, Vero and chick embryo cells, and human embryo fibroblasts (Levkovich *et al.*, 1967). The cytopathic effect (CPE) and plaque formation are variable, a syncytium formation occurs only in certain cells, but a persistent infection is common (Murphy *et al.*, 1995).

Flaviviruses are stable at pH 8.0 but sensitive to temperatures above 40°C, lipid solvents and detergents.

Experimental pathogenicity

TBE virus strains were successfully isolated in suckling laboratory mice and chick embryos. In the pilot studies these hosts were used as a model for studying the pathogenicity of TBE virus strains.

The strains of TBE virus are capable of multiplication in the chick embryo (Slonim and Röslerová, 1965). The pathogenesis of TBE virus in laboratory mice was reviewed by

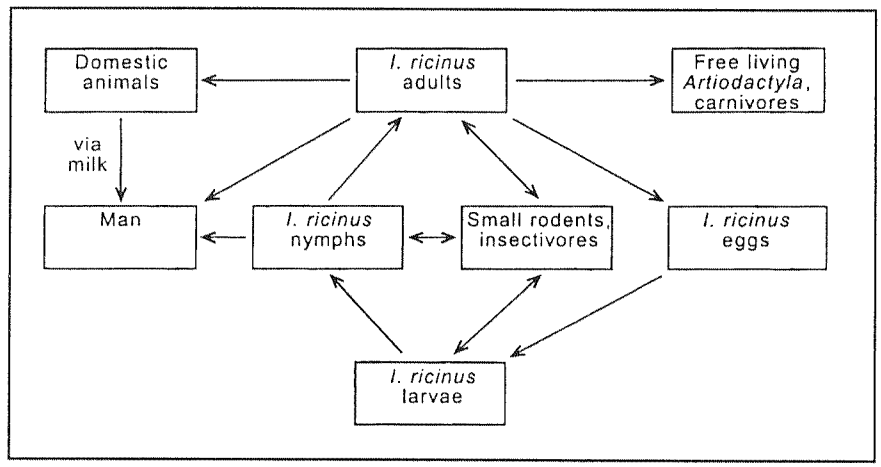

Fig. 2
Circulation of European subtype of TBE virus in the nature

Albrecht (Albrecht, 1962). It is of interest that the immunoflorescence of TBE virus antigen is first observed in scattered symphatetic and parasymphatetic ganglia, sometimes in striated muscle fibres and in some parts of the central nervous system (CNS) in white mice. The fluorescence in the peripheral nerve trunks is observed 1-2 days later. One of the outstanding features of TBE virus infection is the affinity of this virus to the peripheral nervous system (Albrecht, 1962).

Syrian hamsters are susceptible to the virus, however, it takes more virus to kill these animals than to kill mice of the same age. An inapparent infection of adult hamsters was demonstrated by development of antibodies (Slonim *et al.*, 1966a).

After intracerebral (i.c.) and intraspinal (i.s.) inoculation of TBE virus in rhesus monkeys, clinical and histological signs of encephalitis develop (Zilber, 1960; Ilienko and Pokrovskaya, 1960; Slonim *et al.*, 1966b).

After TBE virus administration into subcutaneous tissues, it is transported to blood via lymphatic system (Málková, 1969).

A persistence of RSSE virus in experimentally inoculated monkeys has also been reported (Pogodina *et al.*, 1981). The virus was isolated from monkey tissues by co-cultivation and explantation procedures as late as 383 days after inoculation.

Clinical features

TBE in Europe is a relatively mild clinical disease with low case-fatality rate (Hloucal and Gallia, 1949; Grinschgl, 1955; Grešíková, 1972; Grešíková and Nosek, 1981).

The disease affects all categories of men and women. Typical is its sudden onset preceded by an incubation period of about 7 days. The course is mono- or biphasic. Prodromal symptoms (intracranial hypertension, headache, nausea, vomiting, weakness, loss of apetite, hyperestesia and photophobia) are suddenly developing. There is a rise of temperature to about 38°C, which in 2-7 days disappeares. Sometimes, in the acute phase, certain signs of neurotropism could be observed. There are disturbances in vision (blurred vision) and diplopia. The viraemic phase lasts 4-6 days. Thereafter the symptoms of brain irritation are observed. Objective signs of meningeal irritation with hyperpyrezia and brain involvement with accompanying changes of the internal milieu (principally of the cerebrospinal fluid) are pronounced.

In the second phase, the abovementioned symptoms appear again with pronounced meningo-encephalitis syndromes. According to the symptoms, it is possible to recognize the following types of TBE: abortive, meningeal, encephalitic and encephalomyelitic.

An interesting clinical picture in a patient was observed during the milk-borne epidemic in Rožňava (Blaškovič, 1954). A characteristic sign was hypersomnia, only exceptionally insomnia or inverse type of sleep. Meningeal symptoms formed one of the cardinal features of the disease. Only some cases with paresis of *n. facialis* have been reported. Extrapyramidal and cerebellar syndroms were frequent.

The most characteristic feature of European TBE in comparison with Far Eastern TBE (RSSE) are milder forms and a lower mortality rate. The fatality rate varies from 1-5% and differs in different outbreaks.

Convalescence is usually prolonged; the encephalitis symptoms such as extrapyramidal, vegetative and nuclear lesions persist for longer period.

RSSE differs clinically from European TBE. It is characterized by sudden onset following an incubation period of

10-14 days. The first symptoms are high fever, headache, nausea, vomiting, hyperestesia and photophobia. These symptoms are followed by stiff neck with Kernig's and Brudzinski's signs and pains in trigeminal and paravertebral areas. In some patients, aseptic meningitis is observed while in others an encephalitis is diagnosed. Hemiparesis and hemiplegia appear rarely at the onset of the illness; more often hemiparesis develops after several days. RSSE is frequently complicated by flaccid paralysis with early atrophy and bulbar disturbances. A clinical picture resembling poliomyelitis is observed in about 20% of cases.

In a pilot study the case fatality was 29.2% (Zilber and Soloviev, 1946). In fatal cases, the death occured within the first week after the onset of the disease. Neurologic sequelae were observed in 30 – 60% of survivors, especially residual flaccid paralyses of the shoulders and arms. In other cases, spastic pareses of the lower extremities were reported. RSSE virus can cause a delayed neurological disease, the development of the Kozevnikov's epilepsy (Levkovich *et al.*, 1967). Chronic progressive encephalitis occurring 13 years after infection has been reported (Ogava *et al.*, 1973).

Laboratory diagnosis

An accurate laboratory diagnosis rests on the virus isolation and serological identification. For virus isolation, the material should be taken in the early stage of the illness. Blood should be preferably heparinized or frozen. It is recommended to save the unused tissues frozen at -70°C or on solid carbon dioxide. After bacterial sterility has been proved, the material as blood or spinal fluid may be inoculated without further preparation. Brain tissues or other organs should be suspended in 10% inactivated normal serum free of antibodies or inhibitors using a diluent of neutral or slightly alkaline pH with proteins known to be free of inhibitors. The prepared inoculum should by administered into the animal as quickly as possible. Laboratory mice are highly susceptible to TBE virus after i.c. inoculation and if only one kind of animal is to be used, the baby mouse is that of choice.

Chick embryo cell cultures are useful for isolation of TBE virus, which multiplies there without CPE but produces interferon. This phenomenon can be exploited for establishing the presence of TBE virus (Grešíková, 1972).

The use of cell cultures in the study of flaviviruses (arboviruses) has increased in recent years. Criteria of the virus presence may be CPE, interference, immunofluorescence, haemadsorption or haemagglutination.

A plaque method for virus isolation may be used in special laboratories which have all facilities for this type of work.

Arthropod tissue cultures are known to be highly susceptible to arboviruses. Difficulties in their preparation and maintenance have so far limited their use, but efforts to overcome them are being made in an increasing number of laboratories.

For the identification of individual viruses and strains serological tests are employed. Different types of tests are available for serological diagnosis of TBE virus: virus neutralization (VN), complement-fixation (CF), hemagglutination-inhibition (HI) and enzyme-linked immunosorbent assay (ELISA).

Principally, the VN test is a method of demonstration of virus-inactivating substances present in the serum. With the advances in the preparation of antigens and in the test technique the CF test became very useful diagnostic aid for TBE virus (Casals, 1967). It is necessary to test simultaneously two or more serial blood samples from the diagnosed person. In general, a minimum of 4-fold rise of the titer of CF antibodies is considered significant.

The HI test is of considerable help in virus identification and serological diagnosis of infection. An immunological overlap must be kept in mind in the interpretation of serologic data obtained by these tests. For serological survey, the HI test is of a big advantage (Casals, 1967).

For serological diagnosis of a recent infection the test of choice is the immunoglobulin M antibody capture ELISA (Hoffman *et al.*, 1979; Heinz *et al.*, 1981b).

The most sensitive method for detection of the virus is a reverse transcriptase-polymerase chain reaction (RT-PCR). PCR is a powerful and versatile technique for amplifying of a specific region of a genome using two gene-specific oligonucleotides (primers). RT-PCR appears to offer a specific, sensitive and rapid method for detection of a virus, even in materials not suitable for conventional assays of the infectivity (Saiki *et al.*, 1988; Rotbart *et al.*, 1990; Tse and Forget, 1990).

Ecology

Vectors

Repeated field studies have proved *I. persulcatus* as the principal vector of the Far Eastern subtype of TBE virus (RSSE virus), (Zilber and Soloviev 1946; Levkovič *et al.*, 1967) and *I. ricinus* as the principal vector of the European subtype of TBE virus (Rampas and Gallia 1949; Grešíková 1972; Grešíková a Nosek, 1981).

The seasonal activity of *I. persulcatus* in the Far East of the Russia lasts from the end of April to the beginning of June (Zilber and Soloviev, 1946).

The seasonal activity of nymphs and adults of *I. ricinus* in Europe has two peaks, one in April – May and another

in September – October, depending on the type of natural focus (Grešíková and Calisher, 1988).

In the Hercynic foci, the virus was isolated from nymphs and adults of *I. ricinus*; 0.4 – 0.9% of the nymphs, 1.5 –5% of males, and 5% of females were found infected. In the Carpathian foci, the virus was also isolated from nymphs and adults of *I. ricinus*; 0.3 – 0.8% of nymphs and 0.3 – 6% of adults were found infected. In the Pannonic foci, about 0.1% of nymphs, 0.8 – 2% of male and 0.7 – 3.3 % of female adults were infected (Grešíková, 1972).

As *I. persulcatus* and *I. ricinus* ticks are three-host ticks, their population density depends on the population density of their hosts. The life cycle of *I. ricinus* in the nature extends over about three years. A peak seasonal activity of overwintering nymphs and adults in the Pannonic foci is in early April, in the Carpathian foci in the middle of April, and in the Hercynic foci in early May. The autumn activity peak is lower in Central Europe and only infrequently reaches the values of the spring peak. Enviromental conditions more or less favour the life cycle of *I. ricinus* throughout the Central European temperate zone of TBE virus (European subtype). The transstadial transmission of TBE virus (European subtype) in *I. ricinus* is an important mechanism of virus maintenance (Grešíková, 1972).

I. trianguliceps, *I. hexagonus* and *I. arboricola* are considered secondary amplifying vectors of TBE virus (European subtype) in the nature (Grešíková, 1972). Infected secondary vector species, such as *Haemaphysalis inermis* and *Dermacentor reticulatus* exhibit lower transmission rates. In summary, TBE virus is present in Europe within the range of distribution of *I. ricinus* ticks. A two-peak (spring and autumn) curve of activity of this tick may be related to the human disease. The appearence of virus-infected ticks in spring corresponds to but precedes by a few weeks the period of accumulating human infections.

The distribution of RSSE virus in tissues of *I. persulcatus* ticks was studied experimentally as early as in 1947 (Pavlovskyi, 1941). In female ticks fed on viraemic mice, virus was detected from the 1st to the 25th day post infection (p.i.), in a titer of 10^6 mouse LD_{50}/0.05 ml in the gut, salivary glands and ovaries. The virus was detected in the Malphigian tubules, haemolymph, Gene's organ and brain at low titers.

TBE virus (European subtype) survives in different tick instars for prolonged periods. A persistence of TBE virus in *I. ricinus* for nine months at both 4 °C and room temperature has been documented (Benda, 1958).

In experimental studies, a transstadial transmission of both subtypes of TBE virus has been described. An increase in TBE virus titer was observed after feeding on an infected blood meal; after metamorphosis, a decrease in the virus titer was observed (Benda, 1958). A transovarial transmission of the virus has been demonstrated under laboratory conditions (Benda, 1958; Řeháček, 1962). The success of attempts to demonstrate a transmission may vary according to the level of viraemia in donor host animals and the virus concentration in subsequently gravid females. TBE virus can be transmitted to the vertebrate host by saliva from infected ticks. The highest transmission rates were achieved in adults, followed in order by nymphs and larvae (Benda, 1958).

A sexual transmission of RSSE virus has been demonstrated in *I. persulcatus* (Chunikhin *et al.*, 1983). Infected male ticks transmitted the virus to uninfected females and provided an experimental evidence for transfer of the virus to eggs. Electron microscopy revealed mature virus particles in lumens of endoplasmic reticulum, vacuoles of the Golgi complex of spermatocytes and tubular elements of spermatids.

Under experimental conditions, a replication of TBE virus (European subtype) has been detected in *Ixodes*, *Haemaphysalis* and *Dermacentor* species of ticks; the highest rate of transmission was found in *I.ricinus* ticks (Grešíková and Calisher, 1988).

Hosts

Animals, such as lizards that develop low viraemia, may not circulate the virus significantly but may serve as hosts for ticks and may contribute to the increase and spread of vector populations (Grešíková, 1972).

The experimental pathogenicity of RSSE virus was studied in *Carduelis flammea, C. carduelis, C. spinus, Loxia curvirostra* and *Pyrrhula pyrrhula* (Zilber and Soloviev, 1946). Linnets (*C. flammea*) were found the most susceptible to RSSE virus infection. These birds were infected with large doses of virus, i.e. under conditions not comparable to those found in the nature. An exception to this may be the Omsk haemorrhagic fever caused by a water-borne virus (Kharitova *et al.*, 1985).

The course of experimental infection of bats infected with TBE (Hypr) virus is characterized by long-term viraemia and viral invasion of CNS. Experimentally infected bats sustain infection through long periods in the cold, possibly contributing in this way to the overwintering of the virus (Nosek *et al.*, 1961). A prolonged viraemic infection has been demonstrated also in hibernating dormice and hedgehogs.

TBE virus is maintained in the nature in a cycle involving ticks and wild vertebrate hosts (Fig. 2).

Small rodents and insectivores (shrews, moles and hedgehogs) which have relatively stable population are believed to be important reservoirs of TBE virus (Kožuch *et al.*, 1967; Grešíková and Nosek, 1981).

In carnivores, viraemia has been demonstrated in young foxes (*Vulpes vulpes*), weasels (*Mustela nivalis*) and badgers (*Meles meles*), infected by feeding with TBE virus-

infected nymphs (Radda *et al.*, 1969). An infection of dogs with TBE virus resulted in low viraemia and subclinical infection (Grešiková *et al.*, 1972). However, also a non-viraemic transmission of TBE virus was observed under laboratory conditions (Labuda *et al.*, 1993).

Large mammals, such as goats, sheep, cattle as well as free living artiodactili serve as hosts for adult ticks (Fig. 2).

Epidemiology of TBE

Geographic distribution

TBE occurs in wide area of Europe and former Soviet Union in correlation with the distribution of ixodid tick vector. *I. ricinus* is the main vector of TBE virus in Europe and its typical life cycle ranges from 3 to 6 years. A two-peak curve of *I. ricinus* activity in spring and autumn may be related to the temperature and humidity. The proportion of infected ticks depends on the intensity of viraemia of the host.

In Slovakia, there is a typical seasonal incidence of TBE corresponding to that of ticks. The infection is mediated predominantly by tick bites, while the other modes of transmission are rare (Grešiková *et al.*, 1987).

The geographic distribution of RSSE corresponds to the geographic distribution of *I. persulcatus* ticks (Zilber and Soloviev, 1946).

The highest incidence of RSSE, a severe disease, is in Western Siberia and Ural (Levkovich *et al.*, 1967).

Incidence

TBE is an endemic disease and its morbidity varies in different regions. In Europe, the highest incidence has been found in Czech Republic and Austria. Recently, an increased incidence of TBE was observed in Latvia and Sweeden (Weekly Epidemiol. Record, 1995).

European subtype of TBE virus causes a relatively mild disease with a low fatality rate (Table 1). Its transmission to humans occurs in all age groups having contact with natural foci of TBE virus. Clinically diagnosed outbreaks have been recognized in the Czech Republic (Hloucal and Gallia, 1949; Krejčí, 1949), Slovakia (Blaškovič, 1954), Austria (Grinshgl, 1955; Moritsch and Krausler, 1959; Tongeren *et al.*, 1955), Hungary (Fornosi and Molnar, 1954), Poland (Przesmycki *et al.*, 1954), Russia (Smorodintsev *et al.*, 1953), Bulgaria (Vapcarov *et al.*, 1954), Slovenia (Vesenjak-Zmijanec *et al.*, 1955), Romania (Draganescu, 1964), Scandinavia (Svedmyr *et al.*, 1958; Oker-Blom *et al.*, 1962; Brummer-Korvenkontio *et al.*, 1984; Traavik, 1979), Germany (Sinnecker, 1960; Müller, 1970; Ackermann and Rehse-Kuepper, 1979; Roggendorf *et al.*, 1981), France (Hannoun *et al.*, 1971) Italy (Verani *et al.*, 1979) and Switzerland (Wandeler *et al.*, 1972).

Milk-borne epidemics of TBE occured in the Peterburg (Smorodintsev *et al.*, 1953) and Moscow regions (Drozdov, 1959) in Russia as well as in the Central Europe: in the Rožňava region in Slovakia (Blaškovič, 1954) and in the Styrian region in Austria (van Tongeren *et al.*, 1955). Recently, cases of a family epidemic were described in Slovakia (Žaludko *et al.*, 1994).

TBE (Hypr) virus is excreted in the milk of goats (Grešíková, 1957) and a human infection may result from the consumption of unpasteurized goat's milk.

TBE (Hypr) virus is excreted also in the milk of sheep (Grešiková, 1958a); a human infection may result from the consumption of unboiled sheep milk and its products (cheeses). Sheep milk-borne epidemic of European subtype of TBE virus was recognized in Slovakia (Grešiková *et al.*, 1975).

TBE virus is excreted also by cow milk (Grešíková, 1958b). A cow milk-borne epidemic of TBE was observed in Poland (Jezyna, 1976).

Seasonal distribution

The seasonal appearance of RSSE is limited to the period from May to August. This seasonal incidence is dependent on the temperature and humidity determining ticks development. In Europe, cases of TBE may occur from spring until autumn. Laboratory infections with TBE virus (European subtype) have been reported, mostly acquired by the respiratory route (Grešíková, 1972).

The highest morbidity rate with RSSE was recorded in the 20–40-year age group (Levkovich *et al.*, 1967). The highest morbidity rate with TBE in Europe was recorded in the 15–59-year age group. The data on the age-specific morbidity indicated considerable yearly fluctuation and variation from country to country.

No significant differences in the morbidity rate with RSSE were found between females and males; in Western Siberia, 46% of infected persons were females and 54% were males. A similar situation exists in Europe. The highest proportion in males (56%) was observed in Slovakia in 1984. Data on the sex-specific morbidity indicates the occurence of fluctuation from year to year and from country to country. These differences are likely due to microclimatic differences between the countries.

Control

Control of ticks with DDT and HCH was tested in Russia (Gorčakovskaya, 1957), however, it was not effective in stopping virus transmission in the nature. In Europe, insecticides have not been used on a large scale because of possible undesirable ecological effects.

There is no specific therapy for TBE infection which represents a considerable public health problem in Europe. Therefore a great attention was paid to the developemnt of

preventive measures for protecting people from the disease. Active prophylaxis was applied in the former USSR very soon after the recognition of natural foci of TBE. A formalin- inactivated vaccine prepared from the brain of TBE virus-infected mice was used in mass vaccination (Smorodintsev *et al.*, 1940). The risk of possible allergic reactions led to search for a new type of vaccine prepared from chick embryo tissue cultures (Benda and Daneš, 1961; Levkovich, 1962). An effort was concentrated also on the preparation of a live vaccine from attenuated Langat virus belonging to the TBE virus group (Mayer, 1975).

The most advanced approach to vaccination is that of a cooperative Austrian-British project using virus grown in chick embryo cell cultures, partially purified by chromatography, inactivated by formalin, and applied with $Al(OH)_3$ as adjuvant. Because of side effects, attempts were made to establish a more efficient purification procedure which was achieved by the use of continuous flow zonal centrifugation (Heinz and Kunz,1980). The vaccine produces a serological conversion of recipients (Kunz *et al.*, 1980). It has been demonstrated that the vaccine affords a high degree of protection against the disease which is more than 90 % after two doses and 98-99% after three doses.

An immunoglobulin against TBE virus is used in the specific prophylaxis of TBE (passive immunization). A successful prophylactic measure may be achieved by breaking off the chain of virus circulation. Therefore a vaccination of milking domestic animals was used to prevent alimentary TBE virus infections (Blaškovič *et al.*, 1962).

A specific prevention of milk-transmitted infections and epidemics plays an important role in the prevention of TBE in general. There is a risk that also the dairy products processed from such contaminated milk can be infected with TBE virus. The results of experiments of this kind demonstrated that TBE virus can persist in cream and butter (Grešíková, 1972). The simplest routine way of prevention of TBE spread by crude milk from domestic animals is boiling of milk before consumption.

Investigations on the effect of pasteurization on TBE virus revealed that a pasteurization of infectious milk by the so-called "holding method" (heating at 62-63°C for 30 mins), caused a sharp drop of virus titer but could not guarantee a complete inactivation of the virus. On the other hand, the method of pasteurization at higher temperature (80°C) for a short period of time (1 min) was found to be effective in complete inactivation of TBE virus (Grešíková, 1972).

Also an education of public on conditions leading to infection and preventing bites of *I. ricinus* ticks is of importance (Grešíková and Calisher, 1988). The use of repellents or protective clothing may reduce the risk of tick bites. However, such protective measures are not very practical for persons with frequent exposure to ticks such as persons working in natural foci (e.g. foresters, wood cutters etc.). Health education in utilization of pasteurized or boiled milk is of critical importance in the areas at risk.

Whereas TBE virus cannot be eradicated totally from its natural foci, it can be restricted. Clearing bush and logs from forested areas results in reduction of small mammals and tick populations. This simple procedure considerably changes the characteristic of the habitats of both ticks and their hosts and alters their roles in dissemination of TBE virus (Grešíková, 1994).

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