Tick-borne encephalitis virus infection has been dwelling in Croatia and its worldwide distribution. During the last thirty years, tick-borne encephalitis virus (TBEV) infection has been increasing in many countries of Europe and Asia. There are three subtypes of tick-borne encephalitis virus: European, Siberian and the Far-Eastern subtype. Transmission. In endemic areas, the virus remains in transmissive cycles between Ixodes ticks and small rodents. Clinical picture. In most cases (70–98%) infection goes asymptptomatically. In about one-third of meningitis cases, meningoencephalitis or meningoencephalomyelitis is developed. Postencephalitic syndrome may be the complication of the infection, presenting with neurological symptoms. Diagnosis. Etiologic diagnosis of tick-borne encephalitis virus is only made on basis of laboratory analyses. Reverse transcription-polymerase chain reaction is used for determining the presence of virus in the blood and cerebrospinal fluid. Antibodies in blood and cerebrospinal fluid can be detected by serological tests. Prevention. The most efficient way to control this potentially severe disease with possible serious long-term consequences is vaccination. It should be recommended to persons who live or travel to endemic areas. Conclusion. In Serbia, tick-borne encephalitis virus infection belongs to the list of reportable diseases; however, there are no reported cases because the diagnostics is not performed routinely. We believe that the significance of this zoonosis must be examined in our country and some of its parts because of preliminary positive serological findings found out in Vojvodina as well as because of reported cases in neighboring countries such as Hungary and Croatia and its worldwide distribution. Key words: Encephalitis, Tick-Borne; Tick-Borne Diseases; Encephalitis Viruses, Tick-Borne; Diagnosis; Epidemiology; Disease Transmission, Infectious; Neurologic Manifestations; Vaccination; Endemic Diseases; Zoonoses

**Introduction**

During the last thirty years, tick-borne encephalitis virus (TBEV) infection has been dwelling in natural foci located in wide geographic areas of Japan, China, Russia, south Europe, central Europe and north Europe. The growing number of registered cases of TBEV is the consequence of improving di-
Antigen E has 3 domains: I, II and III, whereby the domain I is responsible for virus adsorption to the cell receptor, while domain II is responsible for endosomal fusion. The bearers of humoral immunity against TBEV are neutralization antibodies against antigen E. The genome encodes 7 non-structural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. NS5 antigen of TBEV plays the role of interferon antagonist [6]. In cell cytoplasm, NS5 binds to prolidase enzyme, which is necessary for maturation of receptors for interferon IFNAR-1. This leads to the loss of IFNAR-1 receptors at the cell surface by which the virus suppress antiviral effect of interferon [7].

There are three subtypes of TBEV: European, Siberian and the Far-Eastern subtype. European subtype is widespread in Northern, Western, Central and Eastern Europe. Siberian subtype circulates in all endemic areas of Russia, whereas far eastern subtype covers China, Japan and Eastern Russia [8]. The co-circulation between various subtypes in the same geographic area is possible [9]. The presence of European and Siberian subtype of virus has been confirmed in rodents Microtus agrestis and Myodes glareolus voles in Finland [10]. The co-circulation between European and Siberian subtype in ticks Ixodes ricinus was verified on the Crimean peninsula in the period from 1980 to 1990 [11]. European subtype of TBEV was verified in ticks Ixodes persulcatus collected from the taigas of the Eastern Siberia. In Siberia, there is the predominance of Siberian subtype, whereas the European subtype is sporadic [12].

The presence of fourth and fifth genotype of TBEV has been found out by using deoxynucleotide probe set. The fourth genotype is represented by the 178-79 isolate from the region of Irkutsk (Russia), whereas the fifth genotype is represented by 10 isolates [9]. Phylogenetic analyses are mostly based on the analyses of E, NS3 and NS5 genes and show the best congruence between the Siberian and Far-Eastern subtype [6].

Transmission

In European woods as well as in Euroasian taiga and in the Far East, the virus is maintained by the transmission between ticks and small rodents. The main vectors are ticks [1]. The absence of enzymes in tick digestive tract makes a tick a suitable species for the transmission of various microorganisms [13]. A well as TBEV, ticks may transmit bacteria, for example, causative agents of Lyme disease and ehrlichiosis/anaplasmosis [14]. Ixodes ricinus is the vector in European countries, Ixodes persulcatus plays the same role in eastern European countries, Russia and the Far East, while Ixodes ovatus serves as a vector in Japan [15]. Ticks become infected by feeding on blood of infected animals during viremic stage. The tick must feed on a vertebrate at least once in each of its developmental stages before its transformation into the next stage. The infection of the tick remains for the whole life [8, 13]. The tick excretes the viruses by saliva and transfers them during the blood meal. All developmental stages, nymph, larva and adult form, may be infected with the virus and are capable of transmitting infection to the humans and animals. Ticks become active at the temperature of 8°C and humidity of 70–80%. European subtype (adult tick) is the most active in the period of May – June and September - October [13].

About one hundred animals (mammals, birds, reptiles) may be infected with TBEV. The primary hosts for the virus in nature are small rodents, in which viremia lasts for 2 – 8 days with high viral load. Larger animals like foxes, rabbits, deer, wild boars, sheep, cattle and dogs do not enable tick infection because viral load following infection of these animals is not sufficiently high for tick to be infected.

Once infected, a tick can infect a human by bite. Tick saliva contains substances which act anti-in-
flamatory, anticoagulant and have an analgesic effect, so a tick bite may go unnoticed [13]. In about one third of confirmed cases, the patients do not remember the tick bite [16]. In endemic areas, humans may be infected by consuming dairy products from non-pasteurized milk of infected animals, especially goats. Outbreaks caused by consuming infected goat milk have been described [17,18]. The human is an accidental host and has no significant role in the virus maintenance in nature. Human-to-human transmission of infection has not been verified.

Pathogenesis

The primary replication occurs at the site of tick bite in Langerhans cells and granulocytes. The virus is spread by the lymph into the regional lymph nodes where it replicates. The virus then reaches various tissues including reticuloendothelial system (liver, spleen, bone marrow) where it replicates intensely. It is neurotropic and affects large neurons of the anterior horns of the spinal cord, medulla oblongata, pons, dentate nucleus, Purkinje cells and the striatum. Spreading to the central nervous system is carried out through the blood-brain barrier by means of incompletely explained mechanism. It is believed that olfactory endothelium and transcytosis play the role in virus transmission to the central nervous system through brain capillary endothelium [15]. The virus spreads through the brain from a cell to a cell per contumtatim.

The infection drives mechanisms of nonspecific and specific immune response. Specific IgM antibodies are detected shortly after infection in serum and cerebrospinal fluid and dwell for at least 6 weeks, while IgG antibodies dwell for lifetime as markers of past infection. Mononuclear cells, mostly CD4+ lymphocytes, CD8+ lymphocytes and, to a lesser degree, natural killer cells and B lymphocytes appear in the cerebrospinal fluid [6]. CD8+ lymphocytes are the most important in the development of cellular immunity.

According to the research results, inflammatory reaction and CD8+ lymphocyte activity lead to neuron impairment with severe consequences. In the research done by Růzek and al., mice with severe immunodeficiency or CD8/- had prolonged survival after being inoculated with neuroinvasive strain of TBEV, that being indicative of immunopathologic mechanisms in the brain damage [19].

Genetic factors in the host and the virus virulence contribute to the development of TBE as well. It is possible that symptomatic forms of TBE are in connection with the deletion of human gene for chemokine receptor CCR5. Namely, it has been found out that a 32-base pair deletion is significantly more common in TBE patients than in the patients with aseptic meningoencephalitis of other etiology [20]. The research performed by Kindberg et al. has shown that functional Toll-like receptor (TLR) is related to TBE [21]. TLR 3 recognizes double-stranded ribonucleic acid (RNA) and it is related to the production of type 1 interferon and inflammatory cytokines like tumor necrosis factor α (TNFα). It has also been shown in the same research that rs3775291 mutation on gene for TLR3 represents a risk factor for encephalitis in humans. It was confirmed earlier that the virus virulence is connected with the gene for E protein of viral envelope. It is now clear that other genes might be connected with the virus virulence as well. By analyzing TBEV isolated from the persons with asymptomatic form of infection, the following three mutations have been discovered: deletion of aminoacid 111 in C protein of capsid; substitution of Ser1534→Phe in NS3 which leads to the mistakes in the assembly of viral particle without RNA and substitution Ser917→Gly which results in substitution of hydrophilic aminoacid, specific for highly virulent strains by hydrophobic acid [22]. Belikov et al. have found that deletions on structural C protein of capsid and substitutions in nonstructural proteins NS3 and NS5 can reduce virulence of TBEV due to disorders in RNA replication and assembly of viral particle and processing of polyproteins [23].

Clinical Picture

The incubation period lasts for 4 – 28 days, usually 7-10 days. It is shorter (3-4 days) in cases of infection transmitted by unpasteurized milk and dairy products. Even 70 – 98% of cases of TBEV infections are asymptomatic [15]. In clinically ill persons infected by European subtype of virus, during viremia, a “flu-like” syndrome without neurological manifestations may develop, lasting for 2 – 7 days. The patient suffers from fever, myalgia, headache and malaise. After the first stage of disease, it may end up with recovery. In about one-third of clinically ill persons, there may be the second stage of disease after afebrile period which lasts for 1 – 20 days [13]. The second stage of disease presents with high-grade fever (>39°C), signs of meningitis, meningoencephalitis or meningoencephalomyelitis. TBE in European countries manifests most commonly as meningitis (50% cases), then as meningoencephalitis in 40% of cases and as meningoencephalomyelitis in 10% of cases. Having followed 1,500 patients infected by TBEV in the period of 1991 – 2000, Keiser reported disturbance of consciousness in 31%, ataxia in 18%, an extremity paresis in 15% and cranial nerve palsy in 11% of patients [24]. The fatal outcome was registered in 1% of patients. In regard to laboratory findings, leukocytosis was registered in 75%, high sedimentation rate in 91%, high level of C-reactive protein in 82% and pleocytosis in cerebrospinal fluid in 100% of patients. Pathologic electroencephalography and magnetic resonance imaging findings were found in 77% and 18% of patients, respectively [25].

Various neurological sequelae, the so-called “post-encephalitis TBE syndrome” remain after 35-58% of
cases of TBE. A prospective study of 124 persons diagnosed with TBE during the 13-year period confirmed post-encephalitis TBE syndrome in 39.5% of cases presenting with spinal nerve paresis/paralysis, hearing impairment, dysarthria and severe mental disorders [26]. In infections caused by Siberian subtype, chronic progressive course of TBE is possible. For endemic area of West Siberia, chronic progressive form of disease was recorded in 1 – 1.7% of TBE cases [27]. The progressive course of disease is considered to be associated with mutations on NS1 gene and inadequate T lymphocyte response.

The mortality and severity of disease depend on the patient’s age and the virus subtype. Older persons are less often asymptomatic and get severely ill more often than children [28]. Infection caused by the Far-Eastern subtype is responsible for monophasic course and the most severe clinical forms of disease. The onset is gradual with fever, headache, loss of appetite, malaise, nausea, vomiting and photophobia, followed by neurological symptoms like extremity paralyses and vision impairment [13]. The Far-Eastern subtype causes severe forms of encephalitis with involvement of the brainstem and the spinal cord. While infections caused by European and Siberian subtype lead to death in about 1%, the mortality in infection caused by the Far-Eastern subtype has reached even 30 – 40% by 1990s. The mortality in TBE caused by the Far-Eastern subtype has been reduced to 13% over the last two decades.

Diagnosis

Etiologic diagnosis of TBE is only made on basis of laboratory analyses, polymerase chain reaction (PCR) test with reverse transcription (RT – PCR) is used for determining the presence of virus in the blood and cerebrospinal fluid. This test has no significance in routine diagnostics because the majority of patients seek medical help when neurological symptoms appear, when viremia is no longer present. This is also the reason why the diagnosis is rarely made by virus isolation. The viremia lasts for about 6 days [29]. For virus isolation biosafety level (BSL) 4 is required.

In addition to neurological symptoms, antibodies in blood and cerebrospinal fluid appear. They can be detected by serological tests, most commonly by enzyme-linked immunosorbent ssay (ELISA) test. IgM antibodies are detectable at the onset of disease (during the first 6 days after the symptoms of encephalitis appeared) and can last for more than 6 weeks of disease. IgM antibodies may appear in the cerebrospinal fluid earlier than in the blood. The highest titer of IgG antibodies can be recorded in the sixth week of disease. Intrathecal IgM antibodies have been detected up to the 6th day of disease in 41% patients, as intrathecal IgG antibodies are detectable between the 21st and 61st day of disease in 98% of TBE patients [30].

The long-term presence of IgM antibodies can be a real problem in result interpretation and evaluation of time of infection. In serological diagnostics, problems arise from cross reactions within the Flaviviridae family [31]. These cross reactions are found in the persons infected with Dengue virus (serotypes 1–4) or those who have been vaccinated against yellow fever or Japanese encephalitis [32]. Consequently, the persons initially or repeatedly vaccinated against TBEV produce IgM and IgG antibodies against TBEV. In fatal cases, TBEV may be isolated or diagnosed with PCR from the brain tissue [33].

Distribution

During the period of 2000 - 2010, 17,741 cases of TBE were reviewed in 30 countries of European Union (EU) and European Free Trade Association (EFTA). Most of the cases were reviewed in the Czech Republic, Lithuania, Latvia and Slovenia. In Europe, the disease was recorded most frequently in males, predominantly in the period of July - October [3]. The European Network for Diagnostics of “Imported” Viral Diseases carried out a study on the distribution of TBE in Europe in the period of 2007 – 2009 and established by interviewing that TBE was on the rise in Austria, the Czech Republic, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Lithuania, Russia, Slovenia, Sweden and Switzerland [34].

Since September 5th, 2012 TBE has been on the list of reportable diseases in EU countries [35]. TBE cases have been recorded in 20 out of 30 members of EU. Four countries have not recorded a single TBE case (Greece, Ireland, Spain and France). In total, 2,560 cases have been recorded. Incidence for EU is 0.52/100,000 residents. The highest incidence (13.35/100,000 inhabitants) in EU was in Estonia in 2012. The incidence in Lithuania and Slovenia was 11.69 and 7.98/100,000 respectively, whereas the incidence in the Czech Republic, Latvia and Sweden ranged from 3 – 5.5/100 000 inhabitants. A low incidence has been recorded in Austria (0.45), Finland (0.72) and Poland (0.31). The lowest incidence was found in Belgium (0.02) and Germany (0.24) [36].

In the period of 1990 – 2009, several thousands of TBE cases were recorded in Russia per year [37]. The decrease in mortality from TBE may be the consequence of the significant improvement in treatment options as well as the appearance of less virulent viral strains.

Treatment

Etiologic treatment does not exist. Therefore, the patients are treated symptomatically, including bed rest in intensive care units at the departments of infectious diseases. Bed rest until a decrease in body temperature and a significant reduction in neurological symptoms are important for better recovery.

Prevention

The best protection against TBEV infection is achieved by vaccination. Vaccines have enabled the
reduction of morbidity in areas with high incidence of TBE [38]. In Sverdlovsk region, Russia, the vaccination has been carried out since 1996 with the resulting decline in the incidence of TBE from 42.1/100,000 in 1996 to 5.1/100,000 in 2006 [16]. In pre-vaccine era, 200-700 TBE cases were reported in Austria per year contrary to 50-100 cases per year after vaccination was introduced within vaccination coverage of 85% [3]. According to Franz et al., the introduction of massive vaccination in Austria has resulted in reduction of TBE incidence by approximately 16% in relation to pre-vaccine era. The incidence remains high for unvaccinated population in Austria [39]. Vaccination is recommended for those living in endemic areas and persons who stay in affected areas for professional, tourism or recreational purposes. Complete vaccination is carried out with three doses followed by booster doses, if needed. According to available literature in English, there are four vaccines applicable in human medicine; two of them are made in EU, and the other two are produced in Russia [16]. The first generation of vaccine was developed in Russia in 1937 by culturing vaccine strains on the mouse brain. Adverse reactions were a significant disadvantage of the first generation – vaccines for TBE. Contemporary vaccines are produced from the whole virion by cultivating on the primary cultures of chick embryo fibroblast cell which are inactivated by formaldehyde. These vaccines are highly refined and contain aluminium hydroxide as adjuvant. A study performed by Leonov on 290 subjects completely vaccinated by commercially available Russian or European vaccines has shown that all vaccines stimulate good humoral immune response and major production of high avidity neutralizing antibodies so that all available vaccines are suitable for mass vaccination against TBEV infection [5, 41].

### Conclusion

In Serbia, tick-borne encephalitis virus infection belongs to the list of reportable diseases; however, there are no reported cases because the diagnostics is not performed routinely. We believe that the significance of this zoonosis must be examined in our country and some of its parts because of preliminary positive serological findings found out in Vojvodina as well as because of reported cases in neighboring countries such as Hungary and Croatia and its worldwide distribution.

### References


