MASARYKOVA UNIVERZITA

TICK-BORNE ENCEPHALITIS

(A Review)

Faculty of Medicine – Masaryk University

Individual Project - VSSP03X

Spring Semester 2019

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Table of content:

1. Abstract 5
2. Definitions 6
2.1. Encephalitis 6
2.2. Meningitis 6
2.3. Aseptic meningitis 6
2.4. Meningoencephalitis 6
2.5. Tick-borne encephalitis 6
3. Epidemiology 7
3.1. Europe 7
3.1.1. Czechia 8
3.2. China 12
3.4. North America 13
4. Etiology 13
4.1. Flavivirus 13
4.2. Tick-borne encephalitis virus 13
4.2.1. Subtypes 13
4.2.2. Structure and genomics 14
4.2.3. Viral replication 15
4.2.4. Mechanisms of dissemination 17
5. Vectors 18
5.1. Other diseases caused by ticks 19
6. Transmission 20
7. Clinical findings 21
8. Diagnosis 22
9. Treatment 22
10. Prevention 23
10.1. Vaccinations 23
10.2. Removing the tick from skin 23
11. Tick-borne encephalitis virus and laboratory safety 24

12. ICD-10 Codes 24
12.1. ICD-10 codes 24
12.2. ICD-11 codes 24
13. Discussion 24
14. Bibliography 25

Acknowledgments

First and foremost, I would like to thank my supervisor, Prof. MUDr. Petr Husa CSc. for his invaluable support and for leading by example. My gratitude is also extended to health care providers at the Infectious Diseases Clinic of the University Hospital in Brno for working miracles.

Last but never the least, to my family, it's a blessing to always have you on my side.

1. Abstract

Tick-borne encephalitis is an arthropod-born disease that involves the central nervous system, caused by tick-borne encephalitis virus (a Flavivirus). It occurs in a seasonal pattern as they are transmitted by ticks; mainly by Ixodes ricinus and Ixodes persulcatus. However, the transmission of the virus by ingestion of unpasteurized milk or milk products have been noted but it accounts for approximately less than 1% of total reported cases.

The disease also affects livestock and birds, and this can potentially cause devastating economical loss.

Clinically, it has biphasic features and many clinical forms; ranging from asymptomatic infection to mild flu-like symptoms and ending with a severe neural manifestations and permanent damage to central nervous system.

For diagnosis, blood serum or CSF sample is used by ELISA to detect immunoglobulins (mainly IgM) against the virus, along with the observation of the clinical manifestations.

TBE is preventable, vaccinations against tick-borne encephalitis virus are widely used with great success in endemic areas. Although, between 10000 to 15000 cases are reported annually. The severity of the disease is generally dependent on age, immunity and the viral subtype. The disease poses greater threat to people living outside urban areas; especially in forests, and to non-immunized travelers coming from non-endemic areas. In Europe, the Baltic states are the most affected by this disease. Anyhow, Czechia have reported a continuous increase of TBE incidence since the mid-1980s.

TBEV is an RNA virus that belongs to the genus Flavivirus. It has three main subtypes; Western, Siberian, and the Far eastern. The latter is the most capable of provoking the severest clinical forms.

Several studies have demonstrated that vectors of TBEV showed obvious sensitivity to climate conditions and for heavy industrial emission of pollutants.

2. Definitions:

2.1. Encephalitis:

"An acute inflammation of the brain. It may be caused by a viral or bacterial infection or it may be part of an allergic response to a systemic illness or vaccination". **[1]**

2.2. Meningitis:

"An inflammation of the meninges due to infection by viruses or bacteria or fungi. Meningitis causes an intense headache, fever, loss of appetite, intolerance to light and sound, rigidity of muscles, especially those in the neck, and in severe cases convulsions, vomiting, and delirium leading to death". **[2]**

2.3. Aseptic meningitis:

"A type of meningitis that encompasses all types of inflammations of the brain meninges other than that caused by pus producing organisms. It is usually a benign illness. Etiology of aseptic meningitis is very wide and includes many infections—both viral and non-viral, drugs, malignancy and systemic illness." **[4]**

2.4. Meningoencephalitis:

"Inflammation of the brain and its membranous coverings (the meninges) caused by bacterial, viral or fungal infection. The disease may also involve spinal cord, producing myelitis with paralysis of both legs, sometimes called meningomyelitis". **[2]**

2.5. Tick-borne encephalitis:

"A seasonal viral illness with usually biphasic course, clinically ranging from benign febrile infection to severe involvement of CNS, which may be even fatal. TBE is caused by flavivirus with primarily neurotropic effect". **[3]**

3. Epidemiology

Tick-borne encephalitis can lead to serious neurologic syndromes that could be fatal. People affected by tick-borne encephalitis virus are distributed in their geographic location from far east Asia, all through Russia, China, central Europe, to the northern parts of Europe, forming endemic patterns over these areas. There are 10000 to 15000 reported cases of TBE in Europe and Asia annually. The majority of cases are found in early twenties to middle-aged adults, and of course there's a risk for unvaccinated populations; especially travelers and people who work outside urban areas. **[11] [10] [21]**

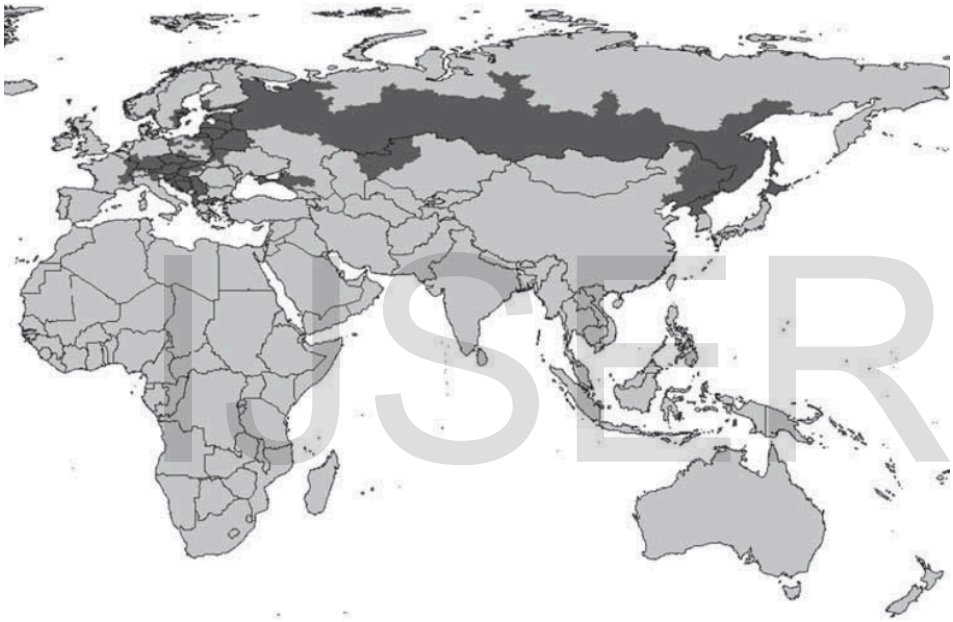


Fig.1 Geographic distribution of TBE. **[10]**

3.1. Europe

"European countries with the highest incidence of the disease in the period 2005-2009 were Slovenia (14.1 cases per 100000 inhabitants per year), Estonia (11.1), Lithuania (10.6), and Latvia (8.8). Pronounced yearly variations of registered TBE cases occurred. According to the latest available epidemiological data for Slovenia the incidence of the disease in 2013 was 15 cases per 100000 inhabitants." **[22]**

A recent study has revealed that between 2012–2016, 23 countries reported 12500 TBE cases but neither Ireland nor Spain have reported any cases. It's also important to note that many countries direct no surveillance to TBE incidents. **[23]**

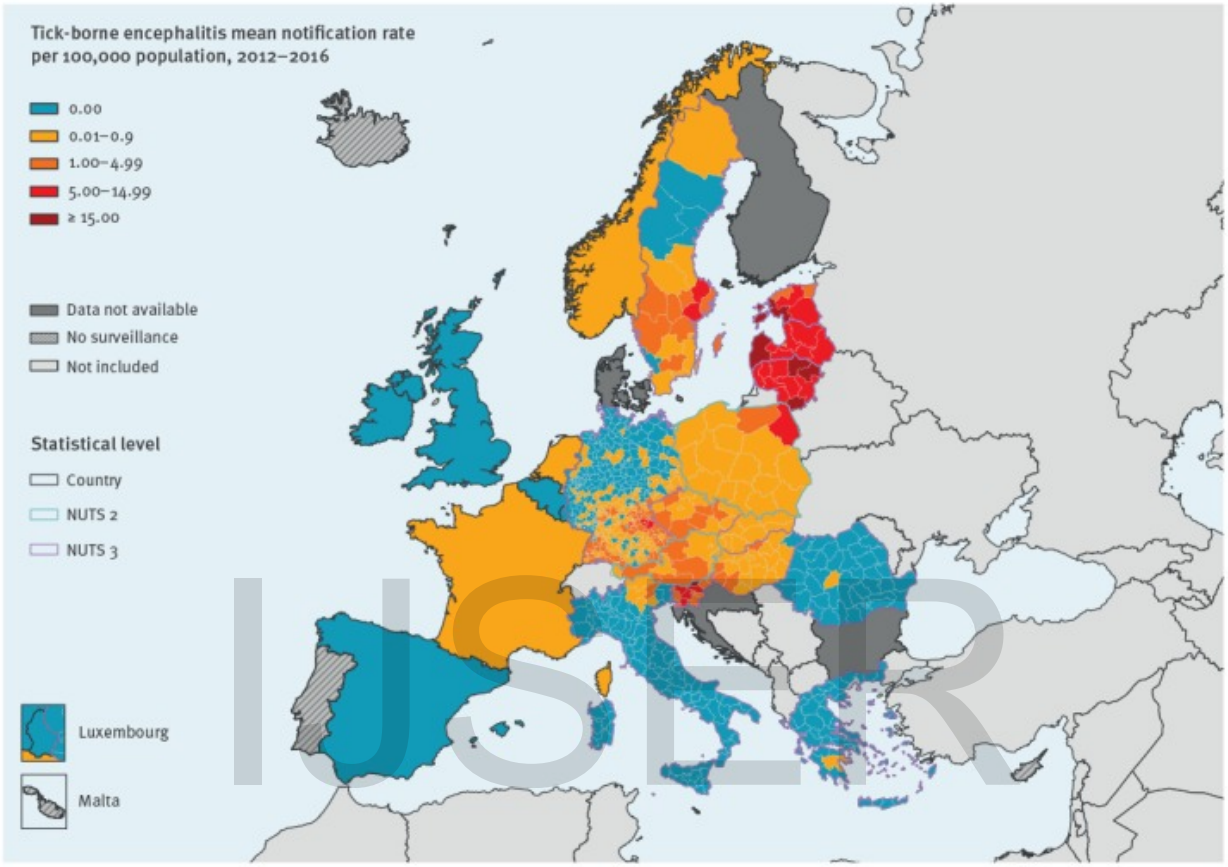


Fig. 2 Rate of locally acquired tick-borne encephalitis per 100,000 population, by place of infection, European Union and European Economic Area countries, 2012–2016 **[23]**

3.1.1. Czechia

Generally speaking, Czechia had a lot less incidences of TBE in the 70s and 80s if compared to the 90s where new reported cases showed dramatic elevation with a peak in 1995 before it sharply decreased again. Nevertheless, there's been a steady rise in the number of incidences since 1985. A shooting increase that reached a peak with a total number of 1029 cases was reported in 2006. This may be correlated to the heat wave that hit Europe in that year. In addition, men are more affected by TBE than women with a ratio of 1.5:1.

Both Czechia and Lithuania make around 38.6% of all reported cases between the year 2012 and 2016, but recent numbers of incidence from Slovenia are alarming as well. **[23] [24] [25] [29]**

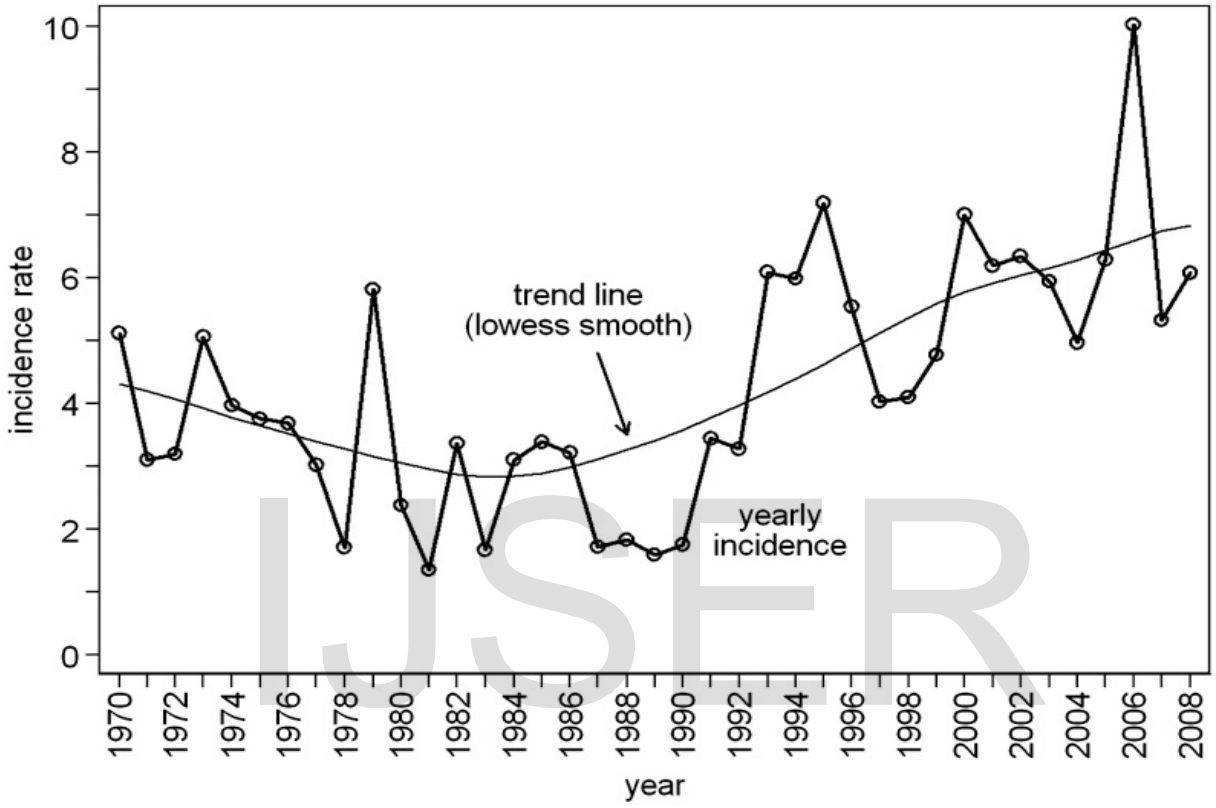


Fig. 3 Tick-borne encephalitis in the Czech Republic 1971–2008, trend in yearly incidence per 100,000 population. **[24]**

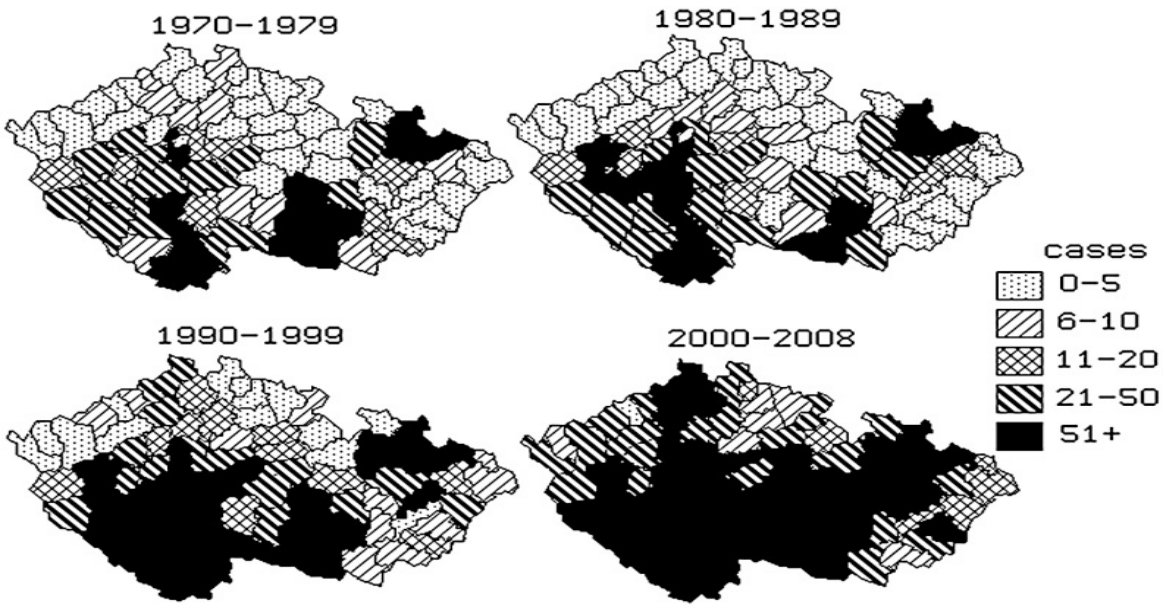


Fig. 4 Reported cases by district of infection and period in Czechia between 1970–2008

Heavy industrial activities in the northern parts of Czechia in the 70s and 80s have resulted in the disruption of northern forests ecosystem and that's why there wasn't much reported for new TBE cases. Nonetheless, with the measures taken to eliminate pollutants emission from factories and the rise of eco-friendlier industrial activity in that region, the ecosystem have been making its way back to equilibrium and Ixodes ricinus thrived again, bringing more TBE cases with it. Currently, the northern parts of Czechia accounts for about 10% of total TBE cases reported from the country.

Number of cases and morbidity per 100 000 inhabitants for tick-borne encephalitis in Czechia between 1999-2018 (source EpiDat/ISIN)*:

year	number of cases	morbidity per 100 000 inhabitants
1999	490	4.77
2000	719	7.00
2001	633	6.19
2002	647	6.34
2003	606	5.94
2004	507	4.97
2005	643	6.28
2006	1029	10.02
2007	546	5.29
2008	631	6.05
2009	816	7.78
2010	589	5.60
2011	861	8.20
2012	573	5.45
2013	625	5.95
2014	410	3.90
2015	355	3.37
2016	565	5.35
2017	687	6.49
2018	712	6.71

* special thanks to Dr. Jan Kyncl from the National Institute of Public Health in Czechia.

3.2. China

In China, tick-borne encephalitis is known as Forest Encephalitis too. It mostly occurs from May to August, similar to the situation in Europe. Currently, there are three main foci where TBE exist in China, the Northeast focus that encompasses Inner Mongolia, Jilin, and Heilongjiang, and Xinjiang focus. There were 2202 cases reported between the year 1980 and 1995, but from 1995 to 1998 there was only 420 reported cases. Statistically, there's peak occurs every 5 – 7 years. **[27]**

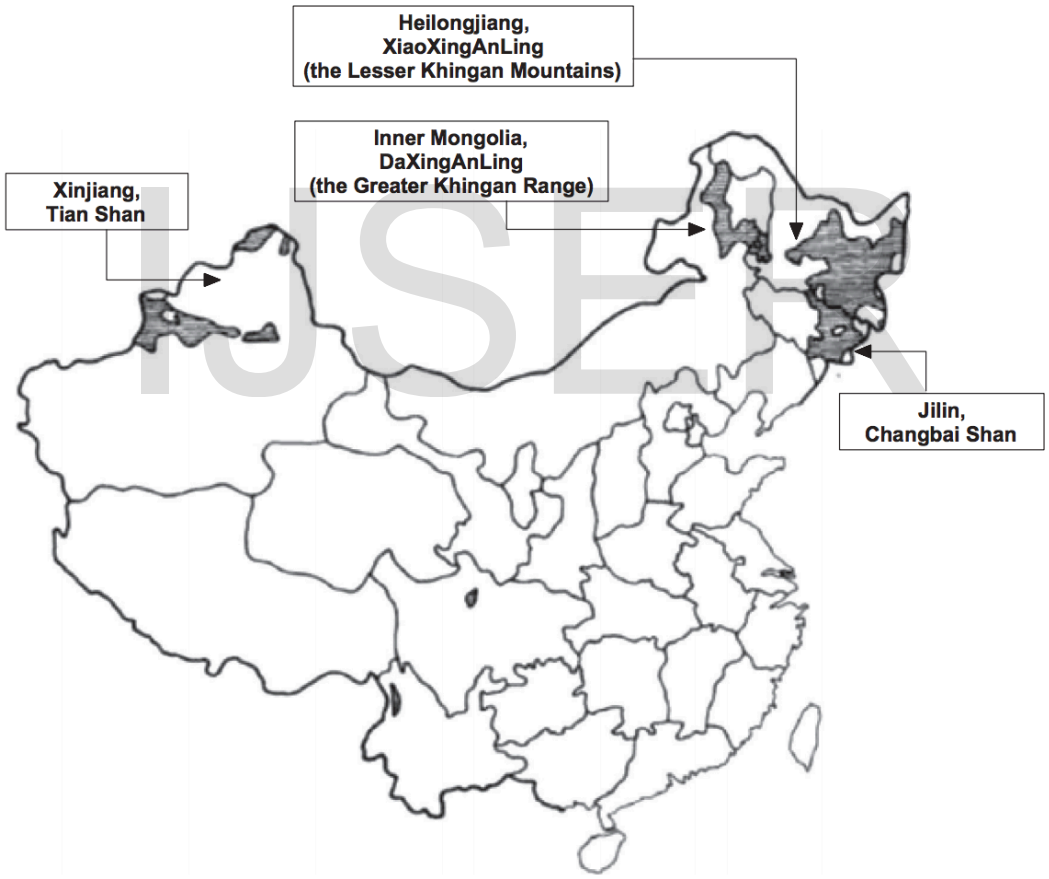


Fig. 5 Geographic distribution of TBE virus infection in China. Shaded areas indicating the natural foci of TBE **[26]**

3.3. North America

Tick-born encephalitis has an extremely rare occurrence in north America; there were only 5 reported cases between the year 2000 and 2009. Those reported cases are highly associated with travelers that spent time in endemic areas in Europe or Asia. Especially if they were not vaccinated. On the other hand, different members of Flavivirus family are more common in north America. **[28]**

4. Etiology

4.1. Flavivirus

Flavivirdae Family includes Flavivirus genus, and the genus includes tick-borne encephalitis virus. Flavivirus takes its name from the Latin word Flavus and it means golden or yellow, this name can be attributed to the yellow fever, it has close to 70 members, 30 of which are known to cause disease to humans, most are arthropod-borne or zoonotic diseases. These genera have no antigenic relationship, even though they're genetically very similar. **[11]**

4.2. Tick-borne encephalitis virus

TBEV is the causative agent for tick-borne encephalitis, an infectious disease of the CNS. It belongs to Flavivirus family so it typically possesses a linear, positive-sense, single stranded, non-segmental RNA. It occurs with an envelope and has an icosahedral shape. Unlike Reovirus and Arenavirus, it doesn't come with a virion-associated polymerase. **[5]**

4.2.1 Incubation period

TBE has a 3 to 14 days incubation period. **[3]**

4.2.2. Subtypes

Based on genetic material sequencing, TBEV has three subtypes:

1- Central European encephalitis (CEE): Also called the western subtype.

2- Russian spring-summer encephalitis (RSSE): Also called far eastern subtype.

3- Siberian subtype: Also called Vasilchenko virus. **[10]**

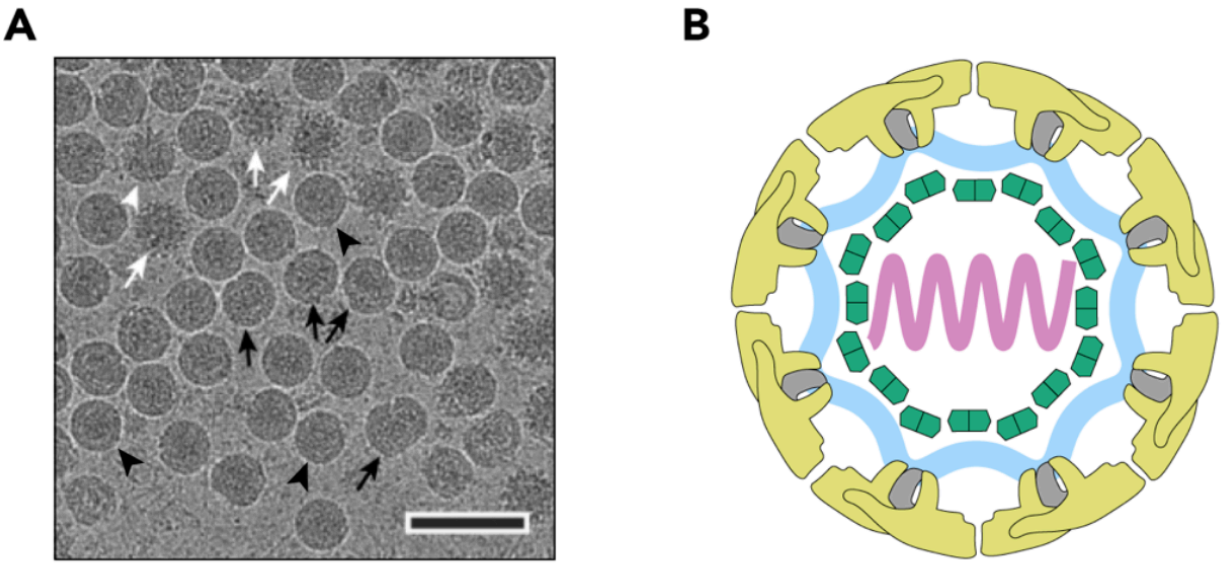
This classification is based on the differences at the amino acid level, there is a 5 – 7% difference between the three subtypes, and even within a single subtype there is a 1.2 – 1.7% difference. **[6]**

Mortality varies depending on the TBEV subtype. An infection with Central European encephalitis subtype has a mortality rate of 1-2%, with death occurring 5–7 days after the onset of the neurological symptoms. The far-eastern subtype usually causes more severe infection with a mortality rate ranging between 5–20%. Chronic or even permanent neuropsychiatric symptoms are seen in 10–20% of patients with tick-borne encephalitis. **[12] [16] [17]**

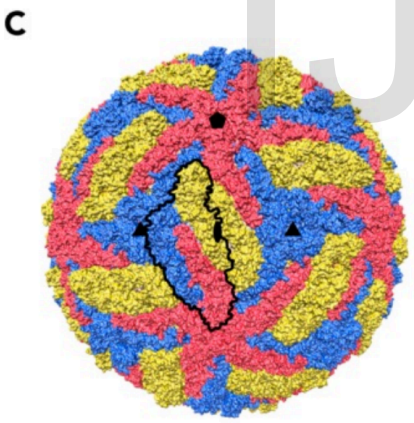
A comparative study has shown no differences in CNS invasiveness between the Siberian and the far eastern subtypes. Although the Siberian subtype exhibited lower infection development rate, it's capable of provoking severe forms of tick-borne encephalitis. **[18]**

4.2.3. Structure and genomics

TBEV has a ~11 kilobase-long positive-strand RNA (+RNA) genome that encodes a single polyprotein that is processed co- and post-transcriptionally into three structural proteins and seven non-structural proteins. Flaviviruses undergo maturation during their production, and infected cells produce at least three types of particles: immature non-infectious particles, partially-mature, and mature infectious particles (Fig. A). TBEV have a 50-nm diameter; another typical characteristic of flaviviruses. The virus is also made of a nucleocapsid and an envelope that is enclosed in a host-cell-derived membrane. The transmembrane domains of the viral envelope and membrane proteins pulls the lipid envelope and giving it a little bit more of an angular shape. The nucleocapsid on the other hand consists of multiple capsid protein copies and a single cope of the genome.



As shown in fig.C, the surface of the TBEV takes an icosahedral asymmetric unit that is outlined in black. The three E proteins within each asymmetric unit are shown in blue, red, and yellow. Symmetry axes are indicated by the black pentagon. **[6,7,8,9]**



4.2.4. Viral replication

Flaviviruses can enter the host cells with the help of its envelope's proteins in three steps; Initially, the virus attaches to the cell membrane. A number of cellular factors were suggested to function as attachment factors or receptors during viral entry, mainly between the virus and cellular glycosaminoglycans. The binding site for these interactions were located on the positively charged surfaces of the E protein. In addition, cellular lectins also increase the efficiency of flavivirus attachment, CD209 Dendritic Cell-Specific Intercellular Adhesion

Molecule-3-Grabbing Non-integrin (DC-SIGN) is an example that is applied for several subtypes of flaviviruses, and These interactions are mediated by N-linked sugars on the prM and E proteins of the virion. Later on, Viruses are internalized via clathrin-mediated endocytosis and fuse with membranes of the late endosome in a pH-dependent manner. Viral mechanism of sensing the surroundings pH is not entirely understood yet, and although several mutagenesis studies of TBEV E proteins have implicated the protonation of His323, possibly His146 too, as the main pH detection mechanism, the cryo-EM reconstruction of the virus implies that other histidines have pH-related roles as well. Afterwards, RNA replication of the microbe starts in association with host cell's plasma membrane and viral particles undergo assembly in the endoplasmic reticulum, viral maturation take place in the acidic golgi apparatus namely by prM protein cleavage by a furin-like protease. **[6]**

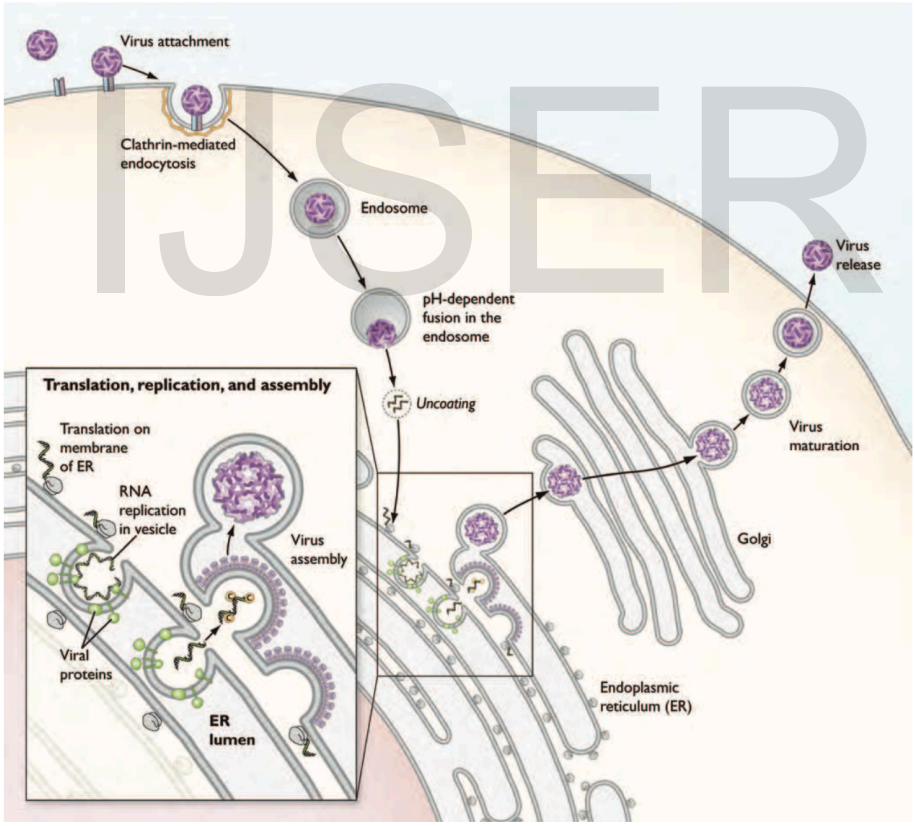


Fig. 6 Viral entry into the cell and viral replication. **[6]**

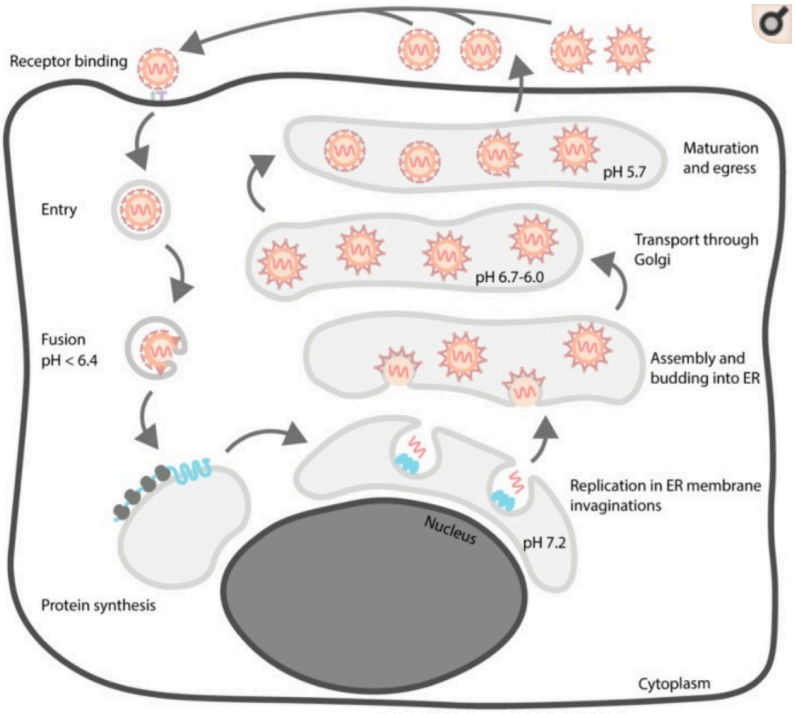


Fig. 7 Viral entry into the cell and viral replication. **[9]**

4.2.4. Mechanisms of dissemination

TBEV is a neurotropic virus and thus requires invasiveness into the CNS and the ability to propagate efficiently within cells of the CNS. In classical studies, it was observed that viruses with low capacity to replicate in the periphery generally had less neuroinvasive potential, regardless of the neurovirulence. Furthermore, data from several studies have shown that time of onset, viremia, capacity of the immune system early reaction influence the risk of entry into the CNS. So, there is a correlation between viral load in the blood and the occurrence of the disease.

Crossing the blood-brain-barrier is likely to be hematogenously; especially if the BBB permeability is increased or if it's functions are disrupted. Other mechanism of CNS invasion includes; direct infectious or passive transport through the endothelium. Gaining access to the CNS though infection of the olfactory bulb since is vulnerable to direct infections is also a possibility. Moreover, a "Trojan horse" mechanism in which the virus is transported to the CNS by infected immune cells. Lastly, direct retrograde transport to the CNS from peripheral neurons in a way that mimics poliovirus mechanism of infection. **[6]**

5. Vectors

Ixodes ricinus and Ixodes persulcatus are responsible for transmission in Europe and Russia, respectively. Ixodes persulcatus are responsible for transmission in Europe and Russia, respectively. These vectors are hard-bodied ticks, that attach firmly to the skin with the help of their strong mouthparts, and a cement-like substance from their salivary glands. This firm attachment ensures a long feeding time that extends from days to weeks if not removed by force. They can reach high number of population in its natural habitat with up to 20% infected ticks per population. Additionally, such vectors can commonly be infected with both Borrelia burgdorferi and tick-borne encephalitis virus and human infection with both diseases have been reported. **[10] [19]**

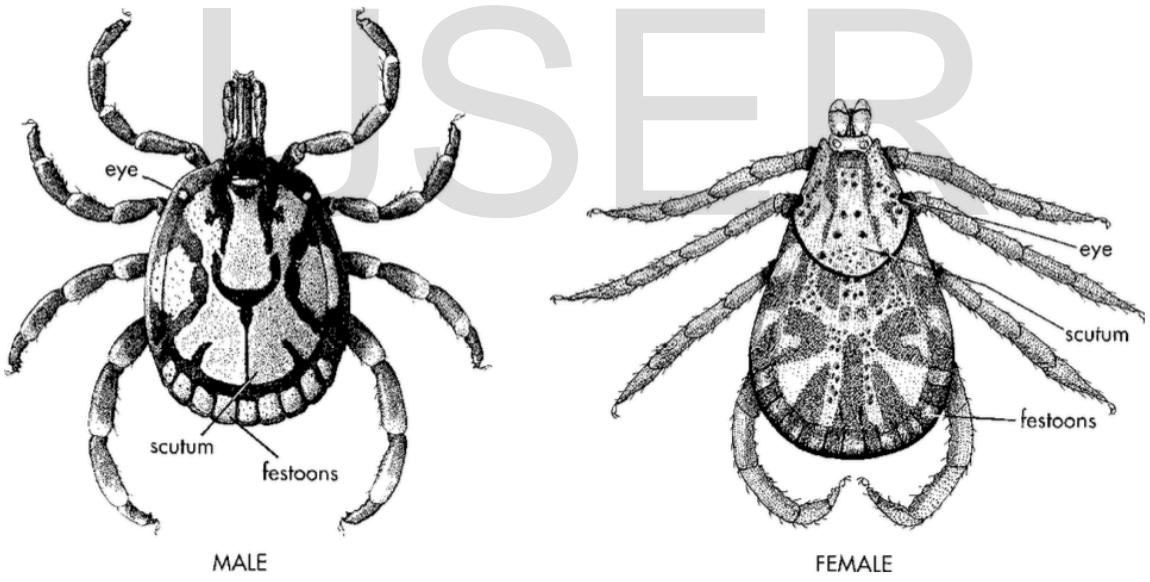


Fig. 8 General appearance of Ixodes ticks (notice the difference in shape in male and female) **[19]**

5.1. Other diseases transmitted to humans by ticks:

Disease	Infective agent	Principal tick vectors	Main reservoir hosts excluding ticks
Tick-borne encephalitis	*Flavivirus*	*Ixodes ricinus*, *I. persulcatus*	Rodents, insectivores
Omsk haemorrhagic fever	*Flavivirus*	*Dermacentor reticulatus*	Muskrats, water voles
Kyasanur Forest disease	*Flavivirus*	*Haemaphysalis spinigera*, *H. turturis*	Monkeys, shrews, rodents
Crimean–Congo haemorrhagic fever	*Nairovirus*	*Hyalomma marginatum* species complex	Hares, cattle, goats
Colorado tick fever	*Coltivirus*	*Dermacentor andersoni*	Many rodent species, rabbits
Rocky Mountain spotted fever	*Rickettsia rickettsii*	*Dermacentor*, *Amblyomma* and *Rhipicephalus* species	Many rodent species
Mediterranean spotted fever	*Rickettsia conorii*	*Rhipicephalus sanguineus*	Rodents, dogs
African tick-bite fever	*Rickettsia africae*	*Amblyomma* species	Rodents, possibly cattle
Q fever	*Coxiella burnetii*	Many ixodid species	Sheep, goats, cattle, possibly rodents
Human ehrlichiosis	*Ehrlichia chaffeensis*	*Amblyomma* and *Ixodes* species	Deer, rodents
Lyme disease	*Borrelia burgdorferi*	*Ixodes ricinus*, *I. scapularis*, *I. pacificus*	Birds, rodents
Tularaemia	*Francisella tularensis*	Many ixodid species	Rabbits, hares, deer, beavers
Tick paralysis	Tick toxins	Mainly *Ixodes* and *Dermacentor* species	Not applicable, as not caused by any pathogen

6. Transmission

TBEVs are transmitted efficiently from infected to uninfected ticks via tick saliva shared during simultaneous feeding on a vertebrate host. RSSE occurs between May and August, whereas CEE cases occur between May and October. **[10]**

Since TBEV infect a wide range of species including birds, rodents, ruminants, and carnivores which make them a reservoir that the virus can be transmitted to humans. The transmission via ingestion of raw milk and or unpasteurized milk products is very possible too. **[12] [13] [14] [15]** The virus can be passed vertically amongst ticks too. **[11]**

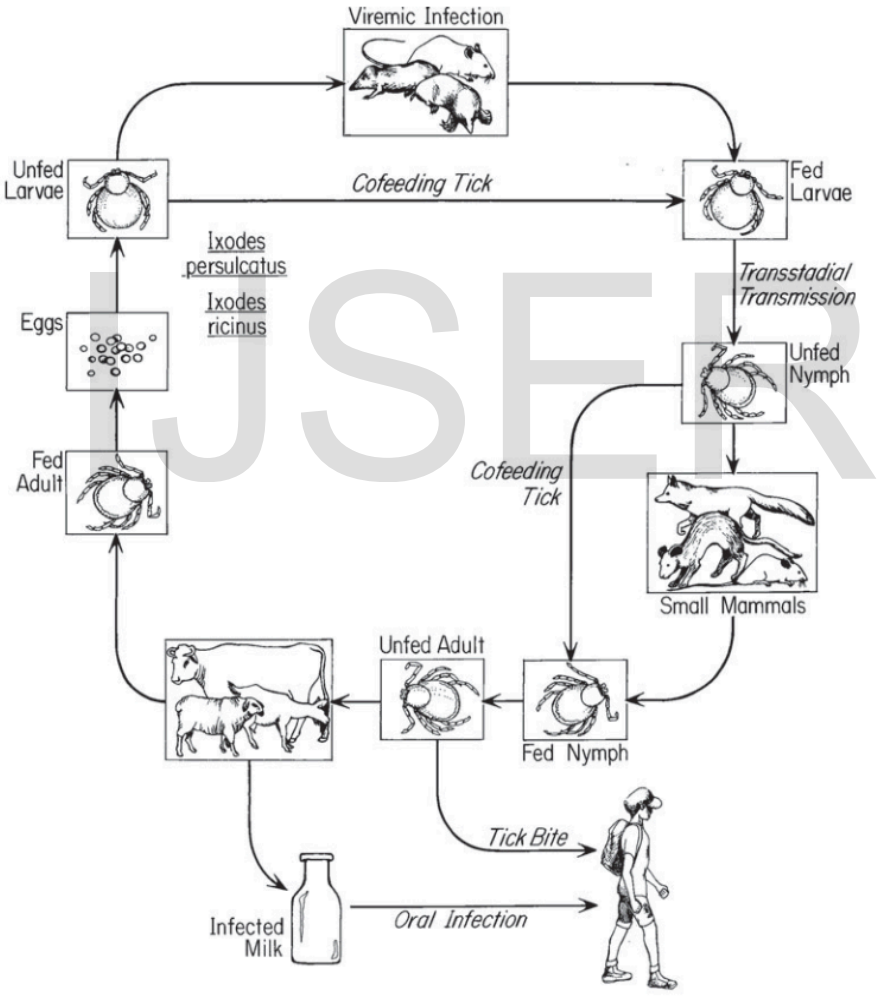


FIGURE 7 Transmission cycle of TBE. Reprinted with permission from T. P. Monath and E. X. Heinz. 1996. Flaviviruses, page 994. In B. N. Fields, D. M. Knipe, and P. M. Howley (eds.), *Fields Virology*, third edition. Lippincott-Raven, Philadelphia, PA.

Fig. 9

7. Clinical findings

Approximately one third of all people that came in contact with an infected tick end up asymptomatic. Otherwise, a biphasic set of symptoms typically take place. Firstly, after the incubation period, the individual develops flu-like symptoms that range from fatigue, headaches, myalgia, arthralgia, fever, nausea. In this phase, the virus can be isolated from the patient's blood. The following second phase corresponds to the viral invasion of the CNS after an asymptomatic period of about one week, and the symptoms include the return of flu-like symptoms along with neurological symptoms as follows;

"1. Febrile syndrome: This illness is characterized by high fever (39°C) with no evidence of neuro-invasion. It lasts from 1 to 5 days, and upon defervescence, patients recover completely.

2. Meningitis. This is the most common form of clinically apparent TBEV infection occurring in approximately 50% of individuals. After the onset of fever, symptoms worsen with progressive headache, nausea, vomiting, and photo- phobia. All patients exhibit a CSF leukocyte pleocytosis after lumbar puncture. Fever lasts 1 to 2 weeks, with gradual recovery.

3. Meningoencephalitis. This form occurs in approximately 10% of cases, is more severe, and is associated with dam- age to the CNS. Individuals become weak, lethargic, and develop focal signs of disease including hemiparesis, hemiplegia, seizures, and autonomic instability. Up to 30% of these cases are fatal, and survivors have long-term neurologic sequelae with slow convalescence.

4. Poliomyelitis-like disease. This is characterized by a prodrome of limb weakness or numbness that progresses to paralysis. Paralysis occurs more frequently in the upper limbs, with the proximal segments affected more often. Recovery is slow, partial, and occurs in only one-half of patients, with the remainder showing progressive deterioration.

5. Polyradiculitis. This syndrome has a biphasic course with fever, headache, and myalgia followed by defervescence. Approximately one week later the second phase starts and is characterized by pain and damage in peripheral nerves, sometimes coupled with meningitis. Recovery from this form of TBEV infection is usually complete.

6. Chronic or persistent infection. This form has been described in Siberia and Far East Russia, although not in Europe, and is believed to associate uniquely with the Siberian subtype of TBEV. Chronic or persistent infection is characterized by a late phase (months or even years later) deterioration of the neurologic sequelae that developed during the acute illness. Alternatively, chronic TBEV infection can begin with the acute phase of disease, such that neurologic symptoms occur years after a tick bite. Clinical symptoms can include epilepsy, Parkinsonian movement and cognitive disorders, and progressive muscle atrophy, ultimately with dementia and death ensuing. Although infectious virus has not been routinely recovered in autopsy studies, a TBEV strain was isolated from a patient who died of a progressive (2-year) form of tick-borne encephalitis 10 years after experiencing a tick bite.

7. Post-encephalitic syndrome. Both retrospective and prospective clinical trials have shown that TBEV infection is associated with a slow recovery period that has considerable long-term morbidity. This post-encephalitic syndrome occurs in approximately 40% to 60% of patients, and includes memory disturbances, headache, and affective and gait disorders. The frequency of these symptoms was proportionately higher in more severe cases." **[6]**

It's also relevant to point out that people infected with the far eastern subtype present with more severe neurologic symptoms. **[11]**

8. Diagnosis.

In the first phase, serologic laboratory findings are usually resulted with thrombocytopenia, leukopenia, a slight increase of liver enzymes, and isolated of the virus during this phase is possible. In the second phase, leukocytosis occurs in the blood and CFS, and the detection of IgM antibodies specific for TBEV using ELISA is possible and with more reliable results than the first phase. Elevated cerebrospinal fluid cell count are noticed too. **[20] [21]**

9. Treatment

Supportive.

10. Prevention

10.1. Vaccines

Immunization is the most successful and protective measure against TBEV infection so far. Nowadays, there are four main vaccines against TBEV and they're widely used in endemic areas. **[30] [10]**

FSME-Immun and Encepur are based on the western subtype of the TBEV and they're produced in Austria and Germany, respectively. They're licensed to be used in adults. Pediatric formulations are available for children under the age of one year. Immunization consists of three doses; the second dose is given within 1 – 3 months and the last one is given is given within 5 – 12 months after the second dose. A booster dose should be given every five years for people under the age of 60 years and every 3 years for people who are older than that. **[30] [10]**

In the Russian federation, TBE-Moscow and EnceVir are produced, and are based on the far eastern subtype. Those are licensed to be used for adult and children above the age of three years old. They have a 3-dose schedule, first two doses are given within 1 – 7 months period while the last dose is given 12 months after the second dose. **[30] [10]**

Currently in China, a second generation of purified inactivated-virus vaccine is manufactures and clinical trials have revealed that it's safer and superior to the first generations due to its greater immunogenicity and the less side effects reported when used. **[31]**

10.2. Removing ticks from the skin

There are several steps that should be followed:

1- Disinfect the area of skin.

2- Using tweezers, grab the tick by its mouthparts and pull away.

3- Disinfect the area of skin again.

4- Wrap the tick in a piece of paper or napkin before disposal. **[32]**

11. Tick-borne encephalitis virus and laboratory safety

According to the Centers of Disease Control (CDC) and National Institutes of Health (NIH), TBEV is classified as Biosafety Level-3 (BSL-3) agent; requiring all personnel at risk to be immunized. **[34]**

When it comes to handling and mailing TBEV cultures it's included in category A under UN number 2814 – Infectious Substance Affecting Humans. **[33]**

12. International Classification of Diseases codes

12.1. ICD-10 codes

A84.0 Far Eastern tick-borne encephalitis [Russian spring-summer encephalitis]

A84.1 Central European tick-borne encephalitis

12.2. ICD-11 codes

1C89 Far Eastern tick-borne encephalitis

1C8A Central European tick-borne encephalitis

13. discussion.

One of the challenges we face is that, there's been a constant rise of incidence number since the mid-1980s. Another issue that needs to be addressed is that there's relatively less written in the literature about TBE than other Flaviviruses.

A serious action on a national level is needed to reduce incidences with TBE. Perhaps an awareness campaign organized by medical students' associations from all medical faculties, social media and video tutorials can be used too, an awareness day, or even pushing to make vaccination against the virus mandatory but there's a question if benefits outweighs the cost of such decision.

14. Bibliography

1. Martin, A. Oxford Concise Medical Dictionary. 7th ed. Oxford. Oxford University Press. (2007), 235.

2. Martin, A. Oxford Concise Medical Dictionary. 7th ed. Oxford. Oxford University Press. (2007), 442.

3. Husa P., Krbková L., editors. Infectious diseases for foreign students. Masarykova univerzita press; Brno, Czechia (2013).

4. Kumar, R. Indian J Pediatr (2005) 72: 57. https://doi.org/10.1007/BF02760582

(Surname, First name initial. Book/Article name (year) edition/issue number: page)

5. Moscatello, K. USMLE. Step 1 Immunology and Microbiology Lecture Notes (2013):381.

6. Lindenbach B.D., Murray C.L., Thiel H.-J., Rice C.M. Flaviviridae. In: Knipe D., Howley P., editors. Fields Virology. Lippincott Williams & Wilkins; Philadelphia, PA, USA (2013).

7. Füzik T, Formanová P, Růžek D, Yoshii K, Niedrig M, Plevka P. Structure of tick-borne encephalitis virus and its neutralization by a monoclonal antibody. Nat Commun. (2018 Jan 30); 9(1):436.

8. Smith TJ, Brandt WE, Swanson JL, McCown JM, Buescher EL. Physical and biological properties of dengue-2 virus and associated antigens. J Virol. (1970 Apr); 5(4):524-32.

9. Lauri I. A. Pulkkinen, Sarah J. Butcher and Maria Anastasina. Tick-Borne Encephalitis Virus: A Structural View. Institute of Biotechnology, University of Helsinki, 00790 Helsinki, Finland. June 2018. https://www.ncbi.nlm.nih.gov/pubmed/29958443

10. C Douglas D. Richman, Richard J. Whitley, Frederick G. Hayden, editors,. Clinical virology. ASM press (2017). 4th ed. Washington, DC.

11. M. Estée Török, Ed Moran, Fiona J. Cooke. Oxford handbook of infectious diseases and microbiology. Second edition. Oxford University Press (2017). Oxford.

12. Kaiser, R. Tick-borne encephalitis. Infect. Dis. Clin. North Am. 22, 561–575 (2008).

13. Balogh Z, Egyed L, Ferenczi E, Bán E, Szomor KN, Takács M, Berencsi G.(2012): Experimental infection of goats with tick-borne encephalitis virus and the possibilities to prevent virus transmission by raw goat milk. Intervirology;55:194-200.

14. Slovakia reports highest occurrence of alimentary tick-borne encephalitis in Europe: Analysis of tick-borne encephalitis outbreaks in Slovakia during 2007-2016.
https://www.ncbi.nlm.nih.gov/pubmed/30012472

15. Family outbreak of alimentary tick-borne encephalitis in Slovakia associated with a natural focus of infection.
https://www.ncbi.nlm.nih.gov/pubmed/8891541/

16. Suss, J. Tick-borne encephalitis in Europe and beyond—the epidemiological situation as of 2007. Eur. Surveill. 13, 2–9 (2008).

17. Kaiser, R. Tick-borne encephalitis: clinical findings and prognosis in adults. Wien. Med. Wochenschr. 162 (2012).

18. Pogodina VV, Bochkova NG, Karan' LS, Frolova MP, Trukhina AG, Malenko GV, Levina LS, Platonov AE. Comparative analysis of virulence of the Siberian and Far-East subtypes of the tick-born encephalitis virus.
https://www.ncbi.nlm.nih.gov/pubmed/15597957

19. Service M. Medical entomology for students. 5th ed. Cambridge University Press. Cambridge (2012).

20. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of High-Consequence Pathogens and Pathology (DHCPP), Viral Special Pathogens Branch (VSPB). March 2014. https://www.cdc.gov/vhf/tbe/diagnosis/index.html

21. Dobler G. Zoonotic tick-borne flaviviruses. Vet Microbiol. 2010 Jan 27; 140(3-4):221-8.

22. Petra Bogovic, Franc Strle. Tick-borne encephalitis: A review of epidemiology, clinical characteristics, and management. World J Clin Cases. 2015 May 16; 3(5): 430-441. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4419106/#B10

23. Julien Beauté, Gianfranco Spiteri, Eva Warns-Petit, and Hervé Zeller. Tick-borne encephalitis in Europe, 2012 to 2016. Euro Surveill. 2018 Nov 8; 23(45). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6234529/

24. Bohumir Kriz, Marek Maly, Cestmir Benes, Milan Daniel. Epidemiology of Tick-Borne Encephalitis in the Czech Republic 1970–2008. Vector Borne Zoonotic Dis. (2012 Nov); 12(11): 994–999. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3491623/

25. J. S. Gray, H. Dautel, A. Estrada-Peña, O. Kahl, E. Lindgren. Effects of Climate Change on Ticks and Tick-Borne Diseases in Europe. Interdiscip Perspect Infect Dis. (2009); 2009: 593232. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2648658/

26.] Bi WM, Deng HP, Bu XY. Regionalisation of natural foci of forest encephalitis. Shou Du Shi Fan Da Xue Xue Bao (J Capital Normal Univ: Nat Sci Ed) 1997;18:100–7 [Chinese].

27. Xian-Bo Wu, Ren-Hua Na, Shan-Shan Wei, Jin-Song Zhu, and Hong-Juan. Distribution of tick-borne diseases in China. Wu et al. Parasites & Vectors (2013), 6:119.

28. Centers for Disease Control and Prevention (CDC). Tick-borne encephalitis among U.S. travelers to Europe and Asia - 2000-2009. MMWR Morb Mortal Wkly Rep. (2010 Mar) 26;59(11):335-8.
https://www.ncbi.nlm.nih.gov/pubmed/20339345

29. Kriz B, Benes C, Danielová V, Daniel M. Socio-economic conditions and other anthropogenic factors influencing tick-borne encephalitis incidence in the Czech Republic. Int J Med Microbiol. (2004 Apr);293 Suppl 37:63-8.

30. World Health Organization. Tick-born encephalitis vaccines.
https://www.who.int/immunization/diseases/tick_encephalitis/en/

31. Xing Yi a , Schmitt Heinz-Josef b, Arguedas Adriano c, Yang Junfeng. Tick-borne encephalitis in China: A review of epidemiology and vaccines. Vaccine. 2017 Mar 1;35(9):1227-1237. doi: 10.1016/j.vaccine.2017.01.015. Epub 2017 Jan 30.
https://www.ncbi.nlm.nih.gov/pubmed/28153343

32. Daniel M. Jak se chránit před napadením klíšťaty. National Institute of Public Health (NIPH). (2007).
http://www.szu.cz/tema/prevence/jak-se-chranit-pred-napadenim-klistaty-1?lang=1

33. Tille, Patricia M. Bailey & Scott's Diagnostic Microbiology. 14th ed. (2017). Elsevier. St. Louis, Missouri.

34. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease, Control and Prevention, National Institutes of Health Biosafety in Microbiological and Biomedical Laboratories. (2009)
https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF