

Background Document on Vaccines and Vaccination against Tick-borne Encephalitis (TBE)*

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I. Introduction

Tick borne encephalitis (TBE) is the most important tick-transmitted neurological disease in Central and Eastern European countries and in Russia. Endemic regions range from Northern China and Japan through far Eastern Russia to Europe (*Gritsun et al., 2003b, Barrett et al., 2008*).

TBE is caused by the TBE-virus (TBEV), a flavivirus, which is transmitted mainly by ixodic tick species and by unpasteurized dairy products, mainly goat milk. TBE morbidity has been increasing over the last decades and the disease is continuously spreading to new, formerly unaffected areas. *Süss (2008)* reports a nearly 400% increase of reported TBE morbidity in Europe between 1974 and 2004 and TBE can now be found in new regions (*Charrel et al., 2004; Süss, 2010*) and at higher altitudes (*Holzmann et al., 2009*). Many factors contribute to this increase: expanding tick populations due to climatic factors (*Randolph, 2009; Randolph, 2010*), social and behavioural changes (*Kriz et al., 2004*), as well as changes in land use and leisure activities (*Sumilo et al., 2008*). Also, reporting of TBE cases has been improved substantially over the years and in 16 countries TBE is now a notifiable disease (*Süss, 2010*). Most likely, however, TBE is still considerably underreported, and in 7 (low-) endemic countries, regular reporting of TBE cases is not required by the health authorities. On average, between 1990 and 2009, nearly 8.500 cases of TBE were reported annually in Europe including Russia, although, with considerable variability in incidence from year to year (*Süss, 2010*).

TBEV is a neurotropic virus that can cause potentially fatal meningitis, encephalitis and/or radiculitis. About one third of infected subjects develop clinical disease; of these, 75% show the classic biphasic picture of an initial short-lasting, flu-like illness starting about 2 weeks after the tick bite, and followed after a few symptom-free days by signs of central nervous system (CNS) involvement (*Kaiser, 1999*). Men are affected twice as frequently as women. Post encephalitic neurologic sequelae, termed “post-encephalitic TBE syndrome” (*Kaiser, 2008*) occur in 35-58% encephalitic cases, with a variety of symptoms and outcomes.

Three main TBEV subtypes, closely related genetically and antigenically, are described: The European, also called Western (TBEV- Eu), the Far Eastern (TBEV- Fe) and the Siberian (TBEV- Sib) subtypes (*Ecker et al., 1999*). The clinical course and the probability of death or severe neurologic sequelae depend on the age of the affected person - severity is increasing with age. Moreover, the clinical outcome may in part depend on the infecting TBEV subtype. Thus, the case fatality rate (CFR) in persons infected with TBEV- Eu or TBEV- Sib rarely exceeds 1% (*Kaiser, 2008*), whereas with the TBE- Fe, CFRs up to 30-40% have been reported (*Mandl, 2005; Lindquist and Vapalahti, 2008*). In Western Siberia, where the TBEV- Sib is prominent, the reported CFR was 2-3% (*Lindquist and Vapalahti, 2008*).

Repellents or insecticides provide unreliable protection against tick-bites (*Ginsberg, 2005*) and specific treatment options are lacking, since there is no antiviral with activity against TBE in vivo.

Active immunization is currently the only option for prophylaxis against TBE. Encepur® and TBE-Immun®, the two vaccines that are manufactured in Western Europe, are based on cell

cultured and inactivated TBEV, adjuvanted to aluminium hydroxide (*Zent and Broker, 2005*). Encepur® and TBE-Immun® are widely used in TBE-endemic EU-countries.

Two Russian vaccines are on the market: TBE Moscow Vaccine® is produced by the federal state enterprise Chumakov Institute of poliomyelitis and Viral encephalitis and EnceVir®, which is produced by the Russian company Microgen in Tomsk (*Leonova and Pavlenko, 2009; Vorob'eva et al., 2007*). TBE Moscow vaccine® and EnceVir® are used in Russia and some neighbouring countries (see Table 9). Like the Western TBE vaccines, they are based on primary cell culture of chicken fibroblasts and are using aluminium hydroxide as adjuvant (*Leonova and Pavlenko, 2009*).

The Western vaccines use strains of TBEV Eu subtype (Neudörfl and K23) which are almost identical in amino acid sequence, while the Russian vaccines are derived from the Sofjin and 205 strains, both belonging to the TBEV Fe subtype (*Vorobyova et al., 2007; Leonova and Pavlenko, 2009*). However, the degree of variation between TBEV subtypes is low with a maximum difference of 5,6% at the amino acid level (*Lindquist and Vapalahti, 2008; Ecker et al., 1999*). Both Western vaccines show cross protection in mice against the other TBEV subtypes, and vice versa (*Holzmann et al., 1992; Hayasaka et al., 200; Leonova et al., 2007a*).

For two decades, ample experience and numerous clinical studies have demonstrated the good immunogenicity, safety, and consistency of the Western TBE-vaccines (*Pollabauer et al., 2010a; Pollabauer et al., 2010b; Loew-Baselli et al., 2006; Loew-Baselli et al., 2009; Rendi-Wagner, 2008; Schoendorf et al., 2007; Zent et al., 2003a; Zent and Broker, 200; Zent et al., 2005*). While no controlled trial with clinical endpoints have been conducted, the field effectiveness of these vaccines reaches more than 97% (*Heinz et al., 2007*), boosting properties are well documented, although the duration of protection beyond five years has not been fully established (*Paulke-Korinek et al., 2009; Rendi-Wagner et al., 2004a; Rendi-Wagner et al., 2004b; Rendi-Wagner et al., 2007*). Furthermore, both vaccines have proved to be safe (*Pollabauer et al., 2010b; Baumhackl et al., 2003; Demicheli et al., 2009; Weinzettel et al., 200; Zent and Broker, 2005*).

Published data on the Russian vaccines are more limited. However, both TBE-Moscow Vaccine®, which was the first cell-derived, concentrated, and purified TBE vaccine licensed in Russia, and EnceVir® have been widely used for many years in their country of origin as well as in some neighbouring countries. The safety and immunogenicity of these preparations have been demonstrated in comparative clinical trials with FSME-Immun (*Pavlova et al., 1999; Leonova and Pavlenko, 2009*). High seroconversion rates following immunization with EnceVir was further demonstrated in a study that used TBE-Moscow vaccine as a reference (*Gorbunov et al., 2002*). Observational studies suggest high field effectiveness.

Being a zoonosis, TBE cannot be easily eliminated from endemic areas. However, the introduction of large-scale vaccination campaigns have proven highly effective in reducing the burden of disease. In Austria, where the vaccination coverage in the general population has reached approximately 90%, the number of clinical cases could be reduced to about 10%, as compared to the prevaccination era (*Heinz, 2008; Heinz et al., 2007; Heinz and Kunz, 2004*,

Kunz, 2003). In most highly TBE-endemic countries, large-scale vaccination campaigns are not implemented, however.

II. TBE and magnitude of public health problem attributable to TBE

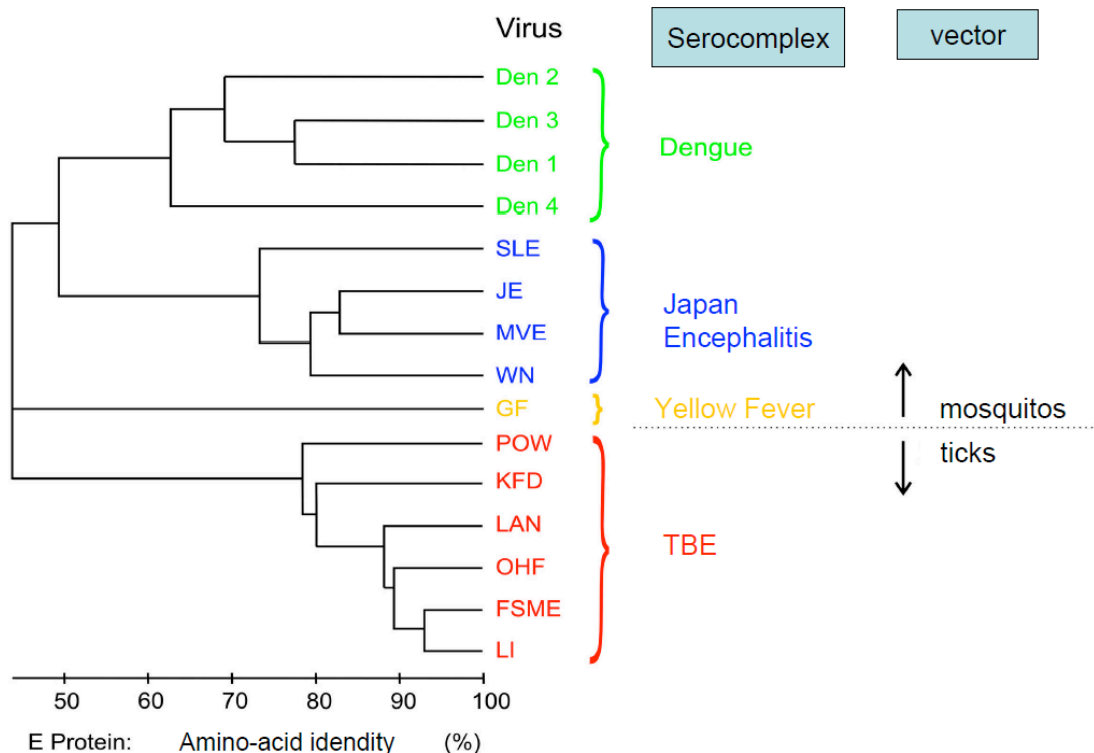
a. Virology

Key points

1. Tick-borne encephalitis virus (TBEV) belongs to the flaviviruses, a large family of enveloped RNA viruses that i.a. also cause Yellow Fever, Japanese Encephalitis, and Dengue fever
2. The viral envelope (E) protein is important for pathogenesis, but is also the major protective immunogen
3. There are 3 closely related TBEV-subtypes: European, Siberian, and Far-Eastern. The subtypes are prevalent in overlapping endemic zones

TBEV is a member of the genus *Flavivirus*, family *Flaviviridae* (Mandl et al., 1997), which comprises about 70 viruses, amongst them the highly pathogenic Yellow Fever, West Nile, Japanese Encephalitis and Dengue viruses. TBEV is a member of the mammalian tick-borne serocomplex, that also includes Omsk hemorrhagic fever virus (OHFV), Langat virus (LGTV), Powassan virus (POWV), Royal Farm virus (RFV), Louping ill virus (LIV), Kyanasur Forest disease virus (KFDV), and the KFDV-subtype Alkhurma hemorrhagic fever virus (AHVF) (Mansfield et al., 2009; Gritsun et al., 2003). TBEV itself has three subtypes: the Western or European-, the Siberian- and the Far Eastern subtype. A phylogenetic tree is shown in Figure 1.

Fig.1: Phylogenetic analysis of mosquito- and tick borne flaviviruses including the TBEV-complex (Heinz and Stiasny, 2010)



The TBE viruses are small, lipid-enveloped viruses, 50 nm in size and with a spherical structure (Mansfield *et al.*, 2009); their genome comprises a single stranded RNA of approximately 11kb (Wengler and Gross, 1978). The single open reading frame encodes 3 structural proteins: The large envelope protein E (a glycoprotein), the core protein (C) and the membrane protein (M) with a molecular weight of 55.000, 15.000 and 8.000 respectively. The E protein is a class II fusion protein. C is the only protein constituent of the isomeric nucleocapsid that contains the virion RNA. The viral genome RNA is infectious (Mandl *et al.*, 1997). The E protein contains the important antigenic determinants responsible for haemagglutination inhibition and neutralization. Furthermore, E protein is the major protective immunogen and the binding of virions to cell receptors is also dependent on this protein (Heinz, 1986).

TBEV is genetically very stable under natural conditions and does not tend to significant antigenic variation. Thus, the three subtypes of the TBEV (European [Eu], Siberian [Sib] and Far East [Fe] subtype) are genetically and antigenetically very similar, although TBEV-Fe and TBEV-Sib are phylogenetically more closely related to each other than to TBEV -Eu (Grard *et al.*, 2007). There is a high degree of homogeneity between different isolates of TBEV-Eu (Holzmann *et al.*, 1992).

Although the endemic zones of the TBEV subtypes can be described separately (see below) it is known that two- or all three subtypes may circulate in the same area at the same time (Golovljova *et al.*, 2004; Demina *et al.*, 2010).

b. Transmission and vector ecology

Key points

1. Ticks are main vectors of TBEV and also serve as virus reservoir; their source of TBEV infection are commonly small rodents
2. Forested areas of Europe and Asia are ideal tick habitats. In recent years, TBE endemic zones have expanded, in part due to climatic changes
3. TBE epidemiology is characterized by the very focal nature of the disease, even in endemic regions
4. Prevalence of TBEV in ticks commonly varies between 0.1% and 5%, but may reach 20-40 %. Infected ticks remain infected for life
5. There is no direct relationship between prevalence of TBEV in ticks and the incidence of TBE in humans
6. The most important tick vectors are *Ixodes ricinus* and *Ixodes persulcatus*. TBEV is contained in the saliva of infected ticks and is transferred when the ticks penetrate human skin for a blood-meal
7. TBE is occasionally contracted through consumption of non-pasteurized milk, usually goat milk

Ticks are the main vectors and serve as virus reservoir whereas vertebrates, mainly small rodents such as the yellow-necked field mouse or voles (*Charrel et al., 2004*) serve as so called amplifying hosts and act as the ticks' source of infection (Large mammals such as roe, deer or goat rarely reach sufficient viremia to be infectious for ticks). In the tick population, TBEV is transmitted by the trans-ovarial route and also by highly effective trans-stadial transmission, that occurs when nymphs and larvae are co-feeding on the same rodent host (*Danielova et al., 2002*). An infected tick remains infected for life.

Ticks become active at temperatures above 8°C and a relative humidity of 70-80%. These parameters are important for tick survival as well as for the seasonality of TBE. Hence, the forested areas of Europe and Asia provide ideal tick habitats (*Gritsun et al., 2003b*).

At least 11 tick species are capable of transmitting TBEV. However, only two species are important vectors:

- *Ixodes ricinus*, the common castorbean tick, acts as principal tick vector for TBEV-Eu in Central and Western Europe, Scandinavia, and in the European part of Russia. TBEV-Eu was isolated also from *Haemophysalis* species on the Korean peninsula (*Ko et al., 2010*)
- *Ixodes persulcatus* is the main vector for TBEV-Sib (in Russia and Finland) and TBEV-Fe (in Russia and Far East Asia (including China and Japan) (*Gritsun et al., 2003b*). *Ixodes ovatus* is transmitting the virus only in Japan.

The increase of TBE cases over the past 2 decades seems to be partly attributable to climatic changes which affects both tick- and rodent populations (*Lukan et al., 2010; Korenberg,*

2009 Randolph, 2009; Onischenko et al., 2007). However, many factors other than altered climate have contributed to the increased disease burden. Those factors include changes in epidemiological, political, social, ecological, economic and demographic conditions, as will be described later on (Suss, 2008, 2010, Sumilo et al., 2007, L'vov and Zlobin, 2007).

In recent years, TBE endemic zones have expanded to higher altitudes. Early studies on epidemiology (Kunz, 2003) defined the upper limit of TBE-occurrence to 800m above sea level. New data show that at least in Austria and Slovakia, TBE may occur at altitudes up to 1.500 m (Holzmann et al., 2009; Lukan et al., 2010).

Recent studies suggest that birds migrating from endemic parts of Russia to Sweden may carry TBEV infected ticks (Waldenstrom et al., 2007). Although birds are not considered to play a major role as reservoir for the virus, their migration may contribute to the dispersal of TBEV to new areas. Evidence for this hypothesis was provided by Golovljova et al., (2004) and by Gould and Solomon (2008). It has been speculated that ticks which feed on both mammals and seabirds may be the evolutionary bridge between mammalian and seabird-borne flaviviruses (Grard et al., 2007).

The prevalence of TBEV in free living ticks is identified by reverse transcription- polymerase chain reaction (RT-PCR). In Europe, this prevalence can vary from up to 1,7% in Lithuania (Han et al., 2005) to 14,3% in a TBEV focus in Switzerland (Casati et al., 2006), but most commonly vary between 0.1% and 5% (Suss et al., 2004). In 2006, surveillance of the tick populations in highly endemic areas of the Russian Federation showed TBEV prevalences that frequently exceeded 10%, and in the Penza Province, 29.2% of the ticks were TBEV-infected (Onischenko et al., 2007). This illustrates the highly focal distribution of the virus even in endemic zones.

TBEV is transferred to the host when the infected tick attaches itself to hair-covered portions of the human dermis and inserts its hypostoma into the punctured skin. As the saliva of the tick is anesthetizing, the bite often passes unnoticed.

A second, but less common way of contracting TBEV is via consumption of non-pasteurized dairy products, especially goat milk. Reports of this route of infections come from Slovakia, Poland, the Baltic states and other Eastern European countries (Kerbo et al., 2005; Vaisviliene et al., 2002; Balogh et al., 2010) and recently, also from a focus >1.500 m above sea level in the Austrian alps (Holzmann et al., 2009). Consumption of raw milk has even caused outbreaks of TBE (Kerbo et al., 2005). Furthermore, TBEV transmission has occurred accidentally in laboratories dealing with this virus. Vertical transmission and transmission via blood transfusion have not been observed in humans.

c. Pathogenesis and pathophysiology

Key points

1. First TBEV replication occurs in dermal cells at site of the bite, then spreads to lymph nodes, followed by viremia and viral dissemination to i.a. CNS
2. In CNS, virus causes inflammation, lysis and cellular dysfunction in particular targeting large neurons of the anterior horns, medulla oblongata, pons, dentata nucleus, Purkinje cells, and striatum
3. Characteristic neuropathologic changes in fatal human cases also include multinodular to patchy polioencephalomyelitis accentuated in spinal cord, brain stem and cerebellum
4. Clinical symptoms of TBE can be explained by affinity of TBEV to distinct regions of the CNS

Following the bite of an infected tick, the first TBEV replication usually occurs locally, in dermal cells. Further replication takes place in the regional lymph nodes; the virus has been found also in Langerhans cells. The affection of lymph nodes is followed by viraemia, during which many extraneural tissues including the reticulo-endothelial system, are infected (*Haglund and Gunther, 2003*). At this stage, the virus also crosses the blood-brain barrier and invades the CNS where it causes inflammation, lysis and cellular dysfunction (*Dumpis et al., 1999; Maximova et al., 2009*). However, it is not fully understood by which mechanisms the acute febrile illness is driven into a severe or even fatal infection of the CNS (*Toporkova et al., 2008*).

The clinical symptoms of TBE can be explained by affinity of TBEV to distinct regions of the CNS (*Maximova et al., 2009*). In lethal cases, common findings include a diffuse lymphocytic infiltration of the meninges and signs of meningitis preferentially in the cerebellum. Also, edematous and hyperemic changes are found in almost all parts of the CNS; the lesions are localized in the grey matter and consist of lymphocytes and lymphocytic perivascular infiltrations as well as of an accumulation of glial cells. Changes in the cerebral cortex are restricted to the motor area with degeneration and necrosis of pyramidal cells (*Kaiser, 2008*). Characteristic neuropathologic changes in fatal human cases also include a multinodular to patchy polioencephalomyelitis accentuated in the spinal cord, brain stem and cerebellum (*Gelpi et al., 2005; Gelpi et al., 2006*). Immunohistochemical visualization of TBEV demonstrates that the virus preferentially targets large neurons of the anterior horns, medulla oblongata, pons, dentata nucleus, Purkinje cells, and striatum (*Gelpi et al., 2005*). There is an inverse topographical correlation between inflammatory change and immunohistochemical detectability of TBEV. Furthermore, a close association between cytotoxic CD8⁺ cells and cell membranes of TBEV-containing neurons has been demonstrated (*Gelpi et al., 2006*).

Studies on experimental TBEV-infection, primarily in mice, suggest that *i)* age is an important determinant for the outcome of the infection, *ii)* persistent infections may occur *iii)* the infection may result in degenerative changes of the CNS (*Mansfield et al., 2009*).

TBEV-Fe and TBEV-Sib have been associated with chronic progressive human encephalitis and Kozhevnikov's epilepsy (Zlontnik *et al.*, 1976; Gritsun *et al.*, 2003a). The former condition represents long-term sequelae of any of the acute forms of TBE, where the neurological symptoms may take years to develop. Another chronic form of TBE is associated with hyperkinesia and an epileptoid syndrome. Hyperkinesia occurs frequently, either during the acute phase or persisting as Kozshevnikov's epilepsy (Mansfield *et al.*, 2009).

d. Disease and disease manifestations

Key points

1. Average incubation period 7-10 days, range 4-28 days. One third of patients bitten by infected ticks develop typical clinical symptoms
2. Of symptomatic patients, 75% develop typical biphasic disease; first phase flu-like illness for 2-7 days without CNS involvement; second phase characterized by high fever and meningitis, encephalitis, and/or radiculitis. Encephalitis is typically dominated by cerebellar signs (ataxia!)
3. Cases of meningoencephalomyelitis contribute 90% of severe sequelae, e.g. pareses of extremities, impaired consciousness, ataxia, pareses of cranial nerves, or need for assisted ventilation
4. In young individuals, meningitis is dominant while with increasing age, meningo-encephalomyelitis is more common
5. In lethal cases, death occurs within 5-10 days of onset of second phase and is mostly associated with diffuse brain oedema and bulbar involvement
6. A postencephalitic syndrome occurs in about 35-58% of TBE patients

About 40-50% of confirmed TBEV-infected patients do not remember any tick bite preceding their illness. After an incubation period of 4-28 days (average 7-10 days), about one third of patients bitten by infected ticks develop typical clinical symptoms (Kaiser, 1999). Length of the incubation period has no prognostic value. Among TBE patients, the ratio of men to women is 2:1.

About 75% of symptomatic patients develop a typical biphasic course of disease with "flu-like illness" during the first viremic phase. Clinical signs include mild to moderate fever, muscle pain, headache and fatigue. This first stage lasts for about 2-7 days without signs of cerebral involvement. Haematologic laboratory parameters show typical signs of a viral infection such as leukopenia, thrombocytopenia and slightly elevated liver enzymes. When recovered from these initial symptoms the patient feels well for about 2-10 days (Kaiser, 1999).

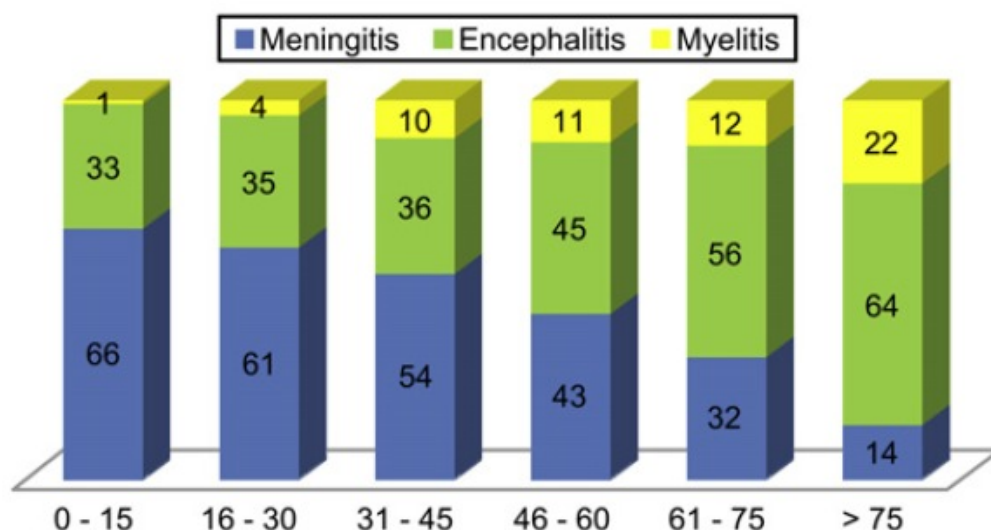
The second phase of the disease is characterized by high fever (often >39°C) accompanied by signs of meningitis, encephalitis, or radiculitis - or mixed neurological forms characterized by

severe headache, stiffness of the neck, nausea, vomiting and vertigo. Pleocytosis is invariably found in cerebrospinal fluid at this stage. Encephalitis caused by TBEV is dominated by cerebellar signs and symptoms typically including ataxia (*Kaiser, 1999*). However, TBEV may induce a variety of neurological symptoms such as disturbed consciousness, convulsions, speech disorder, and vertigo. Affection of cranial nerves with associated symptoms may occur as well.

In cases of meningoencephalomyelitis the affected patient may suffer from paresis of arms, back and legs; the upper extremities more often affected. Encephalitis involving the central brainstem and medulla carries poor prognosis.

In lethal cases, death occurs within 5-10 days of onset of neurologic signs and is mostly associated with diffuse brain oedema and bulbar involvement. Hospitalization may last from 3 to 40 weeks, depending on the severity of illness (*Barrett et al., 2008*), and life-long disabilities may result in very severe, non-fatal cases. CFRs in TBE are possibly associated with the infecting viral subtype: TBE caused by TBEV-Eu will rarely reach more than 1% - 2%, while the TBEV-Fe subtype has been associated with CFRs of up to 30-40% (*Mandl, 2005; Lindquist and Vapalahti, 2008*). However, the high fatality rates associated with TBEV-Fe may be biased by the fact that in most TBEV-Fe endemic areas, access to medical care is limited and therefore, only severely ill patients will be transferred to a clinic. In fact, more recent data suggest that in TBE caused by TBEV-Fe, CFR is 10-20% (*Platonov, personal communication*). As illustrated in Figure 2, the severity of TBE increases with age.

Fig. 2 with permission from *Kaiser, 2008*



It is well recognized that TBE in children below the age of 7 years tends to be less severe, lethality is low, and permanent sequelae occur rarely (*Kaiser, 1999; Kaiser, 2008*). However, in rare cases TBE may take a severe clinical course and result in permanent sequelae also in small children (*Zenz et al., 2005; Jones et al., 2000; Cizman et al., 1999; Schmolck et al., 2005*). In children and adolescents, signs of meningitis are dominant while with increasing

age, combined clinical pictures of meningitis and encephalitis, or meningoencephalomyelitis, are more commonly, and often followed by permanent neurological sequelae.

In about 35-58% of TBE patients a postencephalitic syndrome occurs that consists of a variety of symptoms and signs, such as disturbances of memory, headache, tiredness, hearing impairment, and psychologic disturbances. These problems are mostly transitory, but last more than 3 months in 27% of the cases (*Laurson and Knudsen, 2003; Haglund and Gunther, 2003*). Those suffering from meningoencephalomyelitis contributed 90% of moderate and severe sequelae, such as sustained pareses of extremities, impaired consciousness, ataxia, pareses of cranial nerves, or the need for assisted ventilation (*Kaiser, 1999; Kaiser, 2008*).

e. Immune response

Key points

1. TBE is an immunopathological disease, mediated by inflammatory CD8+ T-cells
2. The exact mechanisms of neuronal death and tissue distruction are still unclear, but inflammation may contribute to neuronal damage
3. Limited data are available on the role of cytokines and chemokines. IgG antibodies confer immunity and persist for a lifetime
4. Neutralizing antibodies represent the most important mechanism of protection against TBEV, as confirmed also by passive transfer experiments in mouse
5. At present, there is no generally accepted quantitative NT- correlate of protection

TBEV- specific cellular and humoral immune responses start to develop shortly after infection. IgM antibodies appear during early stage of the disease, and persist for at least 6 weeks (up to several months), while IgG antibodies tend to appear a little later and to reach maximum concentration in the convalescent phase, around 6 weeks after onset of the disease. IgG antibodies confer immunity and persist for a lifetime (*Holzmann, 2003*). Figure 3 illustrates the antibody dynamics.

Fig 3: Immune response in TBE (with permission adapted after *Holzmann, 2003*).

TBE is an immunopathological disease characterized by an inflammatory reaction mediated y CD8+ T-cells. The inflammation may contribute to neuronal damage and even a fatal outcome (*Gelpi et al., 2005, Gelpi et al., 2006*). The exact mechanisms of neuronal death and tissue distruction are still unclear and limited data are available on the role of cytokines and chemokines. However, patients show elevated levels of TNF- α , interleukin-1 α and IL-6; IL-1 α and TNF- α are acting synergistically in inflammation. IL-10 increases later in the course of

the disease (*Atrasheuskaya et al., 2003*). In contrast to other viral infections only modest increase of IFN- γ are found in TBE patients (*Glimaker et al., 1994*).

Neutralizing antibodies constitute the most important mechanism of protection against TBEV infection, but at present, there is no generally accepted, standardized neutralization test (NT) and no quantitative NT- correlate of protection. Passive transfer experiments in animal (mouse-) models have demonstrated that neutralizing antibodies protect against a lethal TBEV challenge dose (*Kreil et al., 1998*).

f. Diagnosis and differential diagnosis

Key points

1. Clinical diagnosis needs laboratory confirmation
2. PCR- methods are used to detect TBEV in blood and CSF during the first viremic phase of the disease
3. Antibodies against TBEV appear in serum and CSF during second phase of disease; ELISA and NT are commonly used diagnostic techniques
4. Neutralizing antibodies constitute the most important mechanism of protection against TBEV
5. There are a lack of standardized laboratory techniques and no generally accepted serological correlate of protection
6. Serological diagnosis may be misleading due to a) cross-reacting antibodies induced by other flaviviruses; b) persisting IgM against TBEV suggesting TBE in CNS-diseases of other etiologies

Clinically suspected cases of TBE can be confirmed only by laboratory techniques, as the clinical picture of TBE may be similar to that of other viral CNS affections.

TBEV can be detected in blood and CSF during the first viremic phase of the disease using reverse transcription-polymerase chain reaction (RT-PCR) techniques. In practice, these techniques are of minor importance, since patients are mostly diagnosed in the second phase of the disease, following admission to hospital with neurologic symptoms. During this phase the virus has already been cleared from the blood (*Holzmann, 2003*).

During phase 2 of the disease, in parallel with the development of neurological symptoms, antibodies appear in serum and CSF, allowing etiological diagnosis to be confirmed by ELISA (*Holzmann, 2003*). Hemagglutination tests (HI- tests) are rarely used today due to lack of specificity. In the majority of TBE-patients with neurological symptoms, specific IgM and IgG antibodies can be detected in the first serum sample. In the CSF, specific antibodies occur more slowly, but within 10 days of onset of symptoms CSF-antibodies almost invariably become detectable. ELISA tests allowing rapid diagnosis of TBE are commercially available such as Immunozyt FSME® Progen Biotechnik Heidelberg, Germany or Enzygnost® ELISA DADE Behring, Germany. In Russia, Vecto-TBE-IgM and Vecto-TBE-IgG, VectorBest Russia, are available commercially.

Limitations and problems of serologic diagnosis

Persisting IgM antibodies

IgM antibodies may persist for many weeks after TBEV infection or after the first and second TBE vaccination. Without information on previous TBE-vaccinations, positive serological findings caused by recent immunization may lead clinicians to suspect TBE even in cases of other CNS affections.

Cross reactivity:

Individuals who have been exposed to other flaviviruses (e.g. Dengue virus), including those vaccinated against flaviviral diseases (Yellow Fever, Japanese Encephalitis) are likely to show cross-reacting antibodies in TBE-ELISA (IgG) systems. In some cases, positive serological results due to cross-reacting antibodies may have serious clinical consequences. Cross reactivity between antibodies induced by different flaviviruses may be an obstacle also when using ELISA to monitor immunogenicity and duration of the immune response following TBE vaccination.

Where crossreacting antibodies are suspected, there are two options for verification of a possible TBE diagnosis: a) Comparison of ELISA antibody titers in paired sera 14 days apart; if a ≥ 4 -fold increase in antibody concentration occurs, the diagnosis is verified; b) Use of a highly specific TBE-NT assay. In many TBE-endemic regions TBE-NT is not routinely available as this test requires cultivation of TBEV in specialized laboratories with high (L3) biosafety level.

Results obtained by ELISA, HI-tests and NT show excellent correlation with regard to detection of TBEV antibodies, qualitatively as well as quantitatively.

g. Treatment and postexposure prophylaxis

Key points

1. No curative treatment exists for TBE
2. Postexposure prophylaxis using anti-TBEV immunoglobulins is controversial and no longer practised in Western Europe.
3. In contrast, results of early treatment using such immunoglobulins are claimed to be better in Russia, where anti-TBEV immunoglobulins are still available

No curative treatment exists for TBE. Treatment is restricted to symptomatic measures such as antifebrile and antiinflammatory medication (paracetamol, aspirin etc). Corticosteroids are not proven to be of use during the clinical course of TBE. Patients with severe neurologic manifestations have to be closely monitored.

For many years, postexposure prophylaxis (PEP) was performed by application of specific anti-TBEV immunoglobulins (*Broker and Kollaritsch, 2008; Dumpis et al., 1999*). However, this method was never proven to be effective in controlled clinical trials, nor is there sufficient

clinical evidence to support the use of this method. Moreover, it has been suggested that in particular late application of immunoglobulins may aggravate the clinical picture. The evidence for this hypothesis is weak, however (*Arras et al., 1996; Broker and Kollaritsch, 2008*). Immunoglobulin preparations for PEP against TBE were withdrawn from the European market in the late 1990s. In contrast, such products are still used in Russia (*Onischenko et al., 2007*). A recent Russian review concluded that the timely, single administration of one dose (0.05ml/kg body weight) of TBE immunoglobulin with a titre of $\geq 1:80$ ensures protection in on average 79% of the cases (*Pen'evskaya and Rudakov, 2010*). Increasing the dose to 0,1ml/kg, or re-administration of immunoglobulin, provided no additional protection. The conflicting experiences concerning impact of post-exposure immunoglobulin prophylaxis require further analysis.

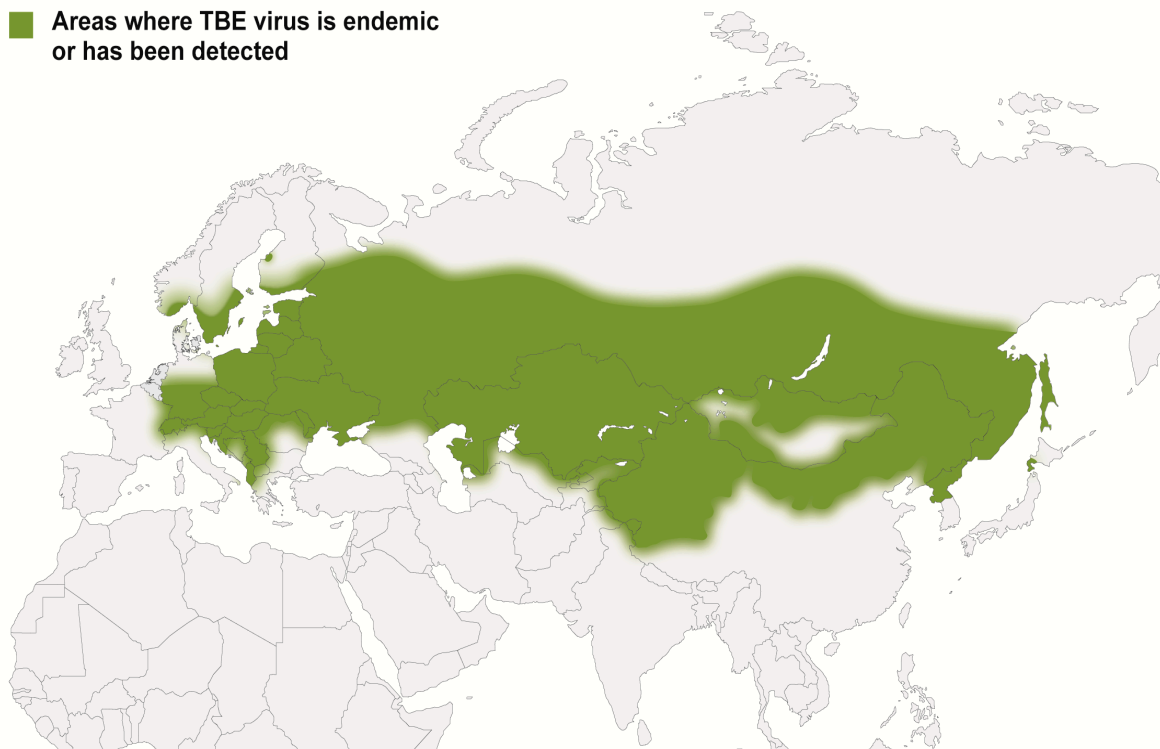
h. *Burden of disease*

Key points (for h. *Burden of disease* and i. *Regional epidemiology and trends*)

1. TBE occurs focally in the non-tropical Eurasian forest belt, most cases occur in Russia and eastern and central parts of Europe.
2. On average, 8500 TBE cases are reported annually; however, the disease is considered underreported
3. On average, 5089 Russian cases annually, overall incidence 1-6 per 100,000; >75% of cases in Siberia and Ural (local incidences may reach >40 per 100,000)
4. European countries with annual incidence >5 per 100,000: Slovenia (14.07), Estonia (11.10), Lithuania (10.59), Latvia (8.76), the Czech Republic (7.02).
5. TBE incidence varies not only between, but also significantly *within* countries
6. No internationally standardized case definitions are available; also, reporting of TBE varies between countries

In endemic areas, TBEV is one of the most important viral causes of meningitis/encephalitis and locally a major public health problem. While globally, on average 8500 cases are reported annually (*Suss, 2010*) this figure is considered an underestimate due to insufficient routine diagnostics and surveillance.

Fig.4 Distribution of TBE and the transmitting ticks (*permission from Petri et al., 2010*)



The distribution of the TBE virus covers almost the entire southern part of the non-tropical Eurasian forest belt, from Alsace-Lorraine in the West to Vladivostok and the northern and eastern regions of China and Hokkaido in Japan in the East (*Barrett et al., 2008; Suss, 2010*).

i. Regional epidemiology and trends

Endemicity has been studied for Central and Western European countries. Two comprehensive publications on the epidemiological situation of TBE were published recently (*Suss, 2008, Suss, 2010*). In brief, between 1990 and 2009 a total of 169.937 cases of TBE were recorded in Europe, i.e. an annual average of 8497 cases. Of these, 2815 cases (33,1%) occurred in Europe excluding Russia. In the period 1976 to 1989, the respective numbers were 2.755 and 1.452 (52%). The reported cases over the last three decades corresponds to an increase of 317,8% in Europe including Russia and 193,2% in Europe without Russia (*Suss, 2008*).

Although there is no doubt that the absolute number of TBE cases has increased over the years, this may in part be attributable to *i*) introduction of mandatory reporting of cases of TBE, *ii*) better surveillance, and *iii*) improved diagnosis, particularly in socioeconomically less developed regions of Europe, where the health systems have improved substantially over a period of 30 years.

Europe:

Currently, TBE is a notifiable disease in most European countries that are considered endemic (see Table 1)

Table 1: Notification of TBE

Countries where reporting of TBE is	
Mandatory	Not mandatory
Austria, Czech Republic, Estonia, Finland Germany, Greece, Hungary, Latvia, Lithuania, Poland, Slovak Republic, Slovenia, Sweden; Norway, Russia, Switzerland	Belgium ^x , France, Italy, Portugal ^x , Spain ^x , Denmark, The Netherlands ^x

^{x)} no autochthonous cases reported

The TBE incidence (i.e. TBE cases per 100.000 inhabitants) varies not only between countries, but also significantly *within* countries. Therefore, the incidence was calculated only in countries where TBE is present in all regions. When considering TBE incidences based on the number of inhabitants in 2008, between 2005 and 2009, European countries can be listed in descending order of TBE incidence, as follows: Slovenia (14.07), Estonia (11.10), Lithuania (10.59), Latvia (8.76), the Czech Republic (7.02), Switzerland (2.15), Sweden (1.99), Slovakia (1.16), Austria (0.94, but approx. 90% of population is already vaccinated!), Poland (0.66), Hungary (0.60), Germany (0.44) and Finland (0.39). Russia has regions of high TBE incidences, but also large non-endemic areas.

The following data are mostly taken from (*Suss, 2008, Suss, 2010*) or referenced separately:

- Austria's incidence data do not reflect the actual situation, since 90% of the population has received at least one vaccination. Current incidence rate is 0,9 per 100,000. Based on studies of field effectiveness of TBE vaccines, incidence would be about ten times higher without vaccination (*Heinz et al., 2007*).
- Croatia has only one natural focus in the northern part of the country. Incidence rates are therefore not calculable. Cases averaged 27 during a period of 5 years between 2003 and 2007 (*Suss, 2008, Suss, 2010*).
- Czech Republic: During the period 2003-2007 an average of 666 TBE cases were reported, peaking in 2006 with 1.029 cases. Incidence is around 7 per 100,000. Spread of TBE to higher altitudes during the last years is reported (*Danielova et al., 2008*).
- Denmark: Only the island of Bornholm with a few cases yearly is considered endemic, but first cases in Denmark outside Bornholm were reported.
- Estonia: With an incidence of 10,4-13,5 per 100,000 during 2003-2007, based on limited data, Estonia is considered to be highly endemic. Consuming unpasteurized dairy products contributes to nearly 30% (in 2005) of all cases (*Kerbo et al., 2005*).
- Finland: Endemic areas are mainly the Åland archipelago (66% reported cases, Incidence 80 per 100,000 in 2000), Turku and Kokkola and Simo, Lappeenranta. Annual reports are around 20 cases.
- France: Single cases around the Alsace region
- Germany: Primarily Southern and Western counties are endemic for TBE, especially Bavaria and Baden-Wuerttemberg, these two states contributing about 85% of all

cases yearly. Germany experiences a steady increase in case reports and a steady expansion of endemic areas northwards and eastwards.

- Greece: was considered to be free from TBE. However, investigation of blood donors revealed seropositivity (ELISA) in 1,6-5,8% (*Pavlidou et al., 2007*), indicating that there may be TBEV activity.
- Hungary: Incidence ranged between 1,3-3,8 per 100,000 until 1996, then a decrease was observed, which may be induced by reduced serological investigation in meningitis candidates (*A.Lakos, personal communication*).
- Italy: Northern Italy is considered to be endemic, case reports are increasing, currently around 20-30 per year.
- Latvia: is a high risk region for both, tick and milk transmitted TBE, incidence ranges between 6,2-10,4 per 100,000 .
- Lithuania: also considered to be highly endemic for TBE, reporting more than 4.500 cases between 1997 and 2008 (*ULAC Centre for Communicable Diseases and Aids. Data report. 2010, accessed via internet*), with an incidence of 11/100,000.
- Kazakhstan: according to *Suss, 2008* endemic regions are located around Almaty, underreporting seems to be common, total number of cases was between 6 and 34 cases annually.
- Norway: TBE was first reported in 1998, southern coastal areas are considered to be endemic. Few case reports.
- Poland: Many small parts of the country are considered to be endemic, mainly the north-eastern parts bordering Lithuania and Belarus and another hot spot are the regions adjacent to the Czech Republic (*Kicman-Gawlowska et al., 2008*). Annual numbers of cases are around 330.
- Romania: Risk areas are considered to be the Tulcea district and Transsylvania. No actual numbers.
- Russia: about 58 million people are currently living in endemic areas. 61.064 cases have been registered between 1998 and 2009, on average 5089 cases per year. Overall incidence rates range between 1-6 per 100,000. Western Siberia is the region with the highest incidence: 40 to >80 x10 per 100,000. Siberia and the Ural mountains together account for >75% of all cases every year. Annual counts of registered cases reach more than 10.000 in some years. Underreporting is, however, strongly suggested, particularly in rural areas. Annual incidence rates tend to vary over the years, the reasons for this phenomenon are unclear.
- Serbia: Few cases in the surroundings of Belgrade, no actual numbers available.
- Slovakia: Most parts of the country are considered to be endemic, annual case numbers range between 46-92 during the period 1998-2007.
- Slovenia: 5 year average was 261 cases between 2001 and 2005, with increasing tendency.
- Sweden: The counties of Stockholm, Södermannland, and Uppsala are considered to be high risk areas, but sporadic cases are being reported from nearly all regions of Sweden. Number of cases is just below 200 per year. There is a trend towards spreading to the western parts of the country.
- Switzerland and Liechtenstein: Switzerland has two high risk regions, the midland (except far western part) and the Rhine valley, including Liechtenstein. Switzerland

registered a continuous increase of cases, peaking in 2006 with 259 cases. The region around Zurich is presently the most endemic one.

- Turkey: no human cases reported
- Belarus, Bosnia, Moldavia, Albania: reporting of cases seems to be not established in these regions, although infected ticks are present.

Regions outside Europe:

- China: TBE is endemic in China, but not a notifiable disease, information is therefore scanty; endemic foci seem to be located mostly in northern and north-eastern parts of the country, for details see (*Lu et al., 2008*). Yunnan and Tibet have a few cases every year. Number of cases seems to be considerable, but absolute numbers are not representative, since TBE is not notifiable and cross reactivity with other flaviviruses, especially Japanese Encephalitis Virus, hampers proper diagnosis.
- Japan: 1 case has been reported, TBEV serosurveys in domestic animals and virus isolations from ticks suggest TBE foci in Hokkaido.
- Mongolia: endemic areas exist close to the Russian border (Selenge and Bulgan, (*Walder et al., 2006; Khasnatinov et al., 2010*).
- South Korea: TBEV-Eu (!) was isolated in several regions from ticks, but no proven human cases are reported so far.

j. Reporting systems and notification

TBE is a notifiable disease across much of Europe, for details see Table 1. However, case definitions vary. Details are given in Table 2 (adapted and reprinted with permission from *Donoso Mantke et al., 2008*).

Table 2.

Member State	Notifiable disease	Case definition	Diagnostic assays	Investigations regarding tick transmitted diseases	Mapping of endemic foci/risk area
Austria	Yes ¹⁾	Serological proven hospitalised TBE cases are counted	ELISA, PCR, NT,	Survey on TBE and rickettsiosis	For human cases
Belgium	No	No	ELISA, PCR	Research project on anaplasmosis, babesiosis, TBE (2007-2010)	In development for human cases, vectors and hosts (rodents, roe deer)
Czech Republic	Yes, since 1971	Clinical and laboratory signs of aseptic meningitis/ meningoencephalitis and positive TBE virus serology	Mostly ELISA, in NRL for arboviruses: CFT and VNT	Tick surveillance in natural foci (TBE and borreliosis)	For human cases and infected ticks
Estonia	Yes, since 1970	Possible case: typical clinical case history (biphasic course of infection), epidemiological links (e.g. tick bite); Confirmed case: with laboratory confirmation: not less than four-fold increase in antibody titre in pair-sera or IgM-antibodies in serum/CSF or positive PCR ⁵⁾	IFA, ELISA, VNT, PCR, SEQ, VI, WB, HIA	Survey on TBE	For human cases
Finland	Yes, since 1996	TBE virus-IgM positive with suitable clinical and anamnestic data (not exposed to other flaviviruses ⁶⁾)	IgM micro-capture ELISA and HIA (PCR only for tick studies)	Tick field surveys (TBE, babesia and anaplasma)	For human cases
France	No	For the diagnosis of TBE, a double check on a pair of serum samples is required (not further specified)	ELISA, VNT only in very few cases (PCR not in routine)	Survey on patients with risk of exposure in infested areas as well as outside	For human cases (only Alsace region)
Germany	Yes, since 2001	Clinical CNS symptomatic case with positive PCR in blood/CSF or IgM- and IgG-antibodies in blood/CSF or increase in IgG-antibody titre or intrathecal antibody production ⁷⁾	ELISA	Tick surveillance (TBE); surveys on borreliosis and rickettsiosis	For human cases
Greece	Yes ²⁾	Clinical CNS symptomatic case with: positive PCR in clinical sample, increased IgG and IgM antibody titres of, IgM detection in CSF, virus isolation	ELISA, IFA, PCR, VI	Survey on TBE (human cases, serosurvey, ticks); survey on CCHF and on bacterial tick-borne diseases	For human cases and ticks, in northern Greece
Hungary	Yes since 1977	Aseptic meningitis, encephalitis or meningoencephalomyelitis confirmed by laboratory tests	IFA, HIA, ELISA	Regular: human cases, serosurvey (TBE); project on tick survey (until 2008)	For human cases and TBE natural foci
Italy	no ³⁾	No	IFA, VI, PCR, micro-neutralisation	not known	For human cases (only north-eastern Italy)
Latvia	Yes, since 1999	No	ELISA	Survey on TBE and borreliosis; tick survey	For human cases and infected ticks
Lithuania	Yes, since 1969	Officially no, but reported cases are serologically proven hospitalised TBE cases	ELISA	Annual tick activity	For human cases
Poland	Yes, since 1970	Clinical description: typical clinical case history (biphasic course of infection); Laboratory criteria: demonstration of four-fold or greater rise of antibody titre in serum or demonstration of intrathecal antibodies or virus isolation from tissues, blood or CSF (for probable case: demonstration of IgM antibodies in serum with no history of previous flaviviral exposure); classification in possible, probable or confirmed cases ⁴⁾	ELISA	Survey on TBE and borreliosis	For human cases
Portugal	No	No	IFA	Survey on rickettsia, borrelia and arboviruses; tick survey	No
Slovakia	Yes, since 1950	Not known	ELISA, HIA (PCR in specific cases)	Survey on TBE and tick survey	No
Slovenia	Yes, since 1977	A case of TBE is considered to be confirmed by the following findings: fever, clinical signs/symptoms of meningitis or meningoencephalitis, an elevated CSF cell count (>5x10 ⁶ cells/L), and serum IgM anti-bodies to TBE virus and/or IgG seroconversion	ELISA, PCR	Survey on human cases and in ticks for TBE, borreliosis, rickettsiosis, anaplasmosis and further tick-borne pathogens	For human cases, ticks and reservoirs
Spain	No	No	ELISA, PCR	Survey on bacterial tick-borne diseases	No
Sweden	Yes ⁸⁾ , since 2004	Under discussion, but reported cases are based on clinical picture and positive serology	ELISA	No	Human cases, incidence
The Netherlands	No	No	ELISA, PCR	Survey on borreliosis (RIVM, Bilthoven)	For borrelia
Norway	Yes, since 1975	No	ELISA	Survey on borreliosis	For human cases, serosurvey in dogs (areas of Kristiansand)
Russia	Yes, since 1950	No formal case definition	ELISA	Survey on human cases and in ticks for TBE, orreliosis, rickettsiosis, CCHF	For human cases and infected ticks
Switzerland	Yes, since 2001	Not known	ELISA	No	For human cases and natural reservoirs

* Data provided by listed contributors.

¹⁾ Notified if meningoencephalitis. Start of notification not further specified.

²⁾ Notification as arboviral encephalitis since 2002 as part of the Commission decision 2002/253/EC.

³⁾ Notification of all acute viral encephalitis cases since 1990. Not specifically TBE.

⁴⁾ Notifiable 1969-1989, and again from July 2004. Voluntary reporting during the period 1990 - June 2004.

⁵⁾ Case definition used since 2004.

⁶⁾ A Baltic/Nordic working group on TBE started in October 2007 to discuss an appropriate case definition.

⁷⁾ Case definition of the Robert Koch Institute according to the Law for the Prevention of Infections (Infektionsschutzgesetz, IfSG), 2007

⁸⁾ Case definition used since January 2005.

The case definitions are partly based on hospitalization, partly on serology, and partly on both. Also, the laboratory diagnosis is not standardized. Some countries do not have an official case definition.

Along with different case definitions, reporting of TBE varies also between countries. For example, in Estonia, general practitioners, hospitals, and laboratories report cases to the regional health boards and these data are then collected by the national health board. In Poland every physician is obliged to report directly to the district's epidemiological centre, these centers report to the Department of Epidemiology at the National Institute of Public Health. In Hungary, all cases of encephalitis have to be reported; in addition, serologically identified cases of TBE are collected by a centralised reference laboratory. In Austria, physicians and hospitals report directly to the Ministry of Health. In Russia every physician is obliged to report directly to the district's epidemiological centre, these centers report to the Federal Service for Supervision of Consumer Rights Protection and Human Welfare.

III. Prophylaxis by vaccines

a. Description of vaccines

Key points (a.Description of vaccines and b. Manufacturing and quality control aspects)

1. Currently, 4 TBE vaccines are licensed: The Western vaccines FSME-Immun® and Encepur® are used mainly in Europe; TBE- Moscow vaccine® and EnceVir® mainly in Russia
2. These vaccines are all based on killed whole TBEV, adjuvanted by aluminium-hydroxide, and are produced according to WHO's Good Manufacturing Practice guidelines. There are some differences in development, preparation and use between Western and Russian vaccines, but differences are limited between the 2 Western vaccines and between the 2 Russian vaccines
3. FSME-IMMUN® is based on the Neudörfl strain, and Encepur® on the K23 strain of the European TBEV subtype. They are produced on chick embryonic fibroblast cells and inactivated by formaldehyde. Pediatric formulation of FSME-Immun® and Encepur® are marketed as FSME-IMMUN (Junior)® and Encepur-K®
4. TBE-Moscow vaccine® is based on the Sofjin strain, and EnceVir on *strain 205* of the Far Eastern TBEV subtype. The viruses are propagated in primary chicken embryo cells and inactivated by formalin. Russian vaccines are not licensed for children <3 years of age
5. Current formulation of TBE- Moscow vaccine® is manufactured since 1999; EnceVir® was first registered in the Russian Federation in 2001

This chapter describes the 4 currently licensed vaccines against TBE:

1. FSME-Immun® (Baxter, Austria)
2. Encepur® (Novartis Vaccines, Germany)
3. TBE- Moscow vaccine® (Federal state enterprise of Chumakow Institute of Poliomyelitis and Viral Encephalitides RAMSci, Moscow)
4. EnceVir® (Scientific Production Association Microgen, Tomsk, Russia)

Vaccines 1 and 2 have undergone central registration by EMA and are available throughout Europe (and in a few countries overseas, e.g. in Canada, but not in the United States); vaccines 3 and 4 are licensed in Russia; TBE- Moscow vaccine is licensed also in Kazakhstan and Ukraine.

All TBE vaccines are based on killed whole TBEV, using Aluminium-hydroxide, Al (OH)₃ as adjuvant. However, as there are differences in development, preparation and use, the vaccines from Western Europe and those from Russia will be described separately.

Encepur® and FSME-Immun®

The two Western vaccines were originally licensed in 1976 (FSME-Immun®) and 1994 (Encepur®); since then they have undergone modifications of production process and composition, as shown in Table 3. This review focuses on current product characteristics, if not stated otherwise.

Table 3: Pharmaceutical composition of widely used tick borne encephalitis vaccines, past and present (Reprinted with permission from *Zent and Broker, 2005*).

	FSME-IMMUN® historic versions (until 2001*)	FSME-IMMUN (new) (since 2001)	FSME-IMMUN Junior (since 2001)	Encepur® historic version (until 1994 ^{‡‡} /2001 ^{§§})	Encepur Adults (since 2001)	Encepur Children (since 2001)
<i>Antigen details</i>						
Strain	Neudörfl	Neudörfl	Neudörfl	K23	K23	K23
Passages	Mouse brain [‡]	PCEC	PCEC	PCEC	PCEC	PCEC
Production	PCEC	PCEC	PCEC	PCEC	PCEC	PCEC
Amount of antigen	1–3.5 µg [§]	2.4 µg	1.2 µg	1.5 µg ^{‡‡§§} /0.75 µg ^{##}	1.5 µg	0.75 µg
<i>Excipients</i>						
Adjuvant	Al(OH) ₃	Al(OH) ₃	Al(OH) ₃	Al(OH) ₃	Al(OH) ₃	Al(OH) ₃
Preservative	Thiomersal [†]	No	No	No	No	No
Stabilizer	HSA [#]	HSA	HSA	Polygeline	Sucrose	Sucrose
Age limit	≥1 year ^{**}	≥16 years	1–15 years	≥1 year ^{‡‡##} / ≥ 12 years ^{§§}	≥12 years	1–11 years
Shelf life ^{**}	18 months	24 months	24 months	18 months	24 months	24 months
Adjusted	0.5 ml	0.5 ml	0.25 ml	0.5 ml	0.5 ml	0.25 ml

* Still available in Czech Republic, Hungary, Russia and Baltic States.

[‡] PCEC for the TBE vaccine Ticovac®, licensed in 2000 in Germany and Austria.

[§] 2–3.5 µg (range) since January 1999.

[†] Removed since January 1999.

[#] Not present in the TBE vaccine Ticovac, licensed in 2000 in Germany and Austria.

^{**} Data pooled from national statistical process controls.

^{‡‡} Formulation for children, adolescents and adults (until 1994).

^{§§} Formulation for adolescents and adults (1994–2001).

^{##} Formulation for children aged 1–11 years (1994–1998).

HSA: Human serum albumin; PCEC: Primary chicken embryonic cells.

FSME-Immun®

Antigen origin and processing

This vaccine was introduced in the 1970s by Immuno AG Austria (subsequently part of Baxter Healthcare) based on the Neudörfl strain of TBEV (*Kunz et al., 1976*). The production of the virus master seed is based on a mouse brain passage of the virus harvested from 5 infected ticks (*Barrett et al., 2008*). The virus from the mouse brain passage is cloned on primary chicken embryo cells (PCEC) and then subjected to 4 further passages in SPF mice to make a master seed. Deriving from this master seed the production virus is propagated on PCEC (*Barrett et al., 2008*). The actual working seeds are subsequently propagated in PCEC.

Earlier versions of this vaccine contained thiomersal. In the late 1990s major modifications were introduced: the seed virus was changed to PCEC, the antigen content was defined in a more narrow range (see Table 3), and thiomersal was omitted (*Zent and Broker, 2005*). All purification and inactivation steps are nearly identical to those of Encepur®.

FSME Immun® contains human albumin as stabilizer. In 1999 an albumin-free TBE vaccine (Ticovac®) was introduced. In clinical studies this vaccine showed an unexpectedly high frequency of acute febrile reactions in children, which was later explained by induction of a high levels of TNF-α (*Marth and Kleinhapfl, 2001*). Therefore, human albumin was reintroduced into the vaccine in 2001.

FSME-Immun® is available in two different formulations, pediatric and adult; the only difference being that the pediatric formulation contains half the adult amount of antigen

(Table3). The pediatric formulation is licensed for children from 1-15 years of age (different from Encepur®); the adult formulation from 16 years onwards.

Encepur®

Antigen origin and processing

Encepur is based on the K23 strain of TBEV. Both the master seed and the working seed are prepared from PCEC cultures. Following propagation in PCEC, the virus is harvested, filtrated and subsequently inactivated by formaldehyde. For concentration and further purification, ultracentrifugation in a sucrose gradient is performed. The final antigen is adsorbed to aluminium hydroxide. Encepur was first licensed in Germany in 1991. Historic versions of Encepur contained polygeline as stabilizer, but preparations since 2001 are free from any preservatives or additives.

When the first pediatric formulation of a polygeline containing vaccine was introduced in 1994, infrequent (approximately 1/50.000) acute allergic reactions were seen in vaccinees. According to postmarketing surveillance in Germany, (*Zent and Hennig, 2004*), these reactions were presumably attributable to polygeline. This led to recall of the pediatric formulation in 1997 and to licensure of a new formulation of Encepur without polygeline as stabilizer in 2001. In the new formulation, a higher concentration of sucrose rendered another stabilizer unnecessary (*Zent et al., 2003a, Zent et al., 2003b*).

The historic versions of Encepur were licensed for both children and adults. However, in 1992 postmarketing surveillance in Germany revealed an increased incidence of adverse reactions in children as compared to adults (*Zent and Broker, 2005*); children suffered more often from febrile reactions, particularly after the first vaccination, and more often the younger the child. A new dose finding study demonstrated that half the amount of antigen was sufficient for an appropriate immune response in children, and that adverse reactions (especially fever) occurred less frequently with the new formulation (*Girgsdies and Rosenkranz, 1996*).

In 2001, the manufacturer introduced separate formulations for children and adults, both formulations polygeline free (*Zent and Broker, 2005*), the only difference between the formulations being the amounts of antigen per dose (see Table 4). The pediatric formulation is licensed for children aged 1-12 years, the adult formulation for individuals ≥ 13 years of age.

TBE-Moscow vaccine®

The TBE-Moscow vaccine® was originally licensed in Russia in 1982 for vaccination of adults. Since 1982, more than 25 million people have been immunized with this vaccine. In the 1990s, the producer improved the purification process to remove heterologous proteins, following which the vaccine was approved for paediatric use. Since 1999 the current formulation (see Table 4) is used.

Table 4: Current pharmaceutical composition of Russian TBE vaccines
(adapted after *Il'chenko et al., 2009, and Vorob'eva et al., 2007*)

Ingredient	TBE vaccine of the Federal state enterprise of Čumakov Institute, Russian Academy of Medical Sciences, Russian Federation (Moscow)	Encevir, Microgen Corporation, Virion Corporation, Ministry of Health, Russian Federation (Tomsk)
TBE strain	Sofjin (Fe)	Strain 205 (Fe)
Passages	Mouse brain	?
Production	PCEC	PCEC
Antigen content	0,5-0,75 µg	2,0-2,5µg
Formaldehyde (mg/dose)	-	-
Aluminium hydroxide (mg/dose)	0.27-0.53	0.3-0.5
Sucrose (mg/dose)	37.0-38.0	20-30
Human albumin (mg/dose)	0.2-0.3	0.20-0.25
Bovine serum albumin (BSA) (µg/dose)	<0.5	-
Chick-embryo protein (µg/dose)	Not specified	<0.5
Protamine sulfate (µg/dose)	<5.0	<10.0
MID 50 (<0.0125 ml)	0.006±0.001	0.005±0.001
Other process-related impurities	Gelatin 5±0.5 µg	-
Shelf life	3years	2 years

Antigen origin and processing

The TBE Moscow vaccine uses a Far Eastern TBEV strain (Sofjin) as source of antigen. The virus master seed is obtained following viral passages in mouse brain; for production the virus is propagated in PCEC (*Vorob'eva et al., 2007*). The main production steps of TBE-Moscow vaccine and Western TBE vaccines are similar (Figure 5). However, with the TBE Mosow vaccine the cell substrate is cultivated in suspension or as monolayers. Following standard manufacturing practices, the harvested virus suspension is inactivated with formalin, then filtrated, concentrated, treated with protamine sulphate, and subsequently gel-filtrated. After addition of 250µg human serum albumin and 5mg gelatine and 37,5mg sucrose per final dose, the vaccine is lyophilized. The vaccine contains additional excipients: protamine sulfate (up to µg per dose), buffer salts, sucrose, chicken albumin (< 0,5 µg per dose) and ≤ 0,5µg bovine serum albumin (BSA). The solvent contains aluminium hydroxide as gel, implying that adsorption takes place after reconstitution of the lyophilized vaccine. Antigen content is 0.5 – 0.75 µg per dose.

Only one formulation is available, and the same dose is administered to adults and children older than 3 years of age.

EnceVir®

Antigen origin and processing

This vaccine was registered in 2001. It is based on the TBEV-Fe strain 205, originally recovered from ticks of the species *Ixodes persulcatus* and deposited in the Tarasevich National Control Institut for use in vaccine production (Patent USSR 669742. Strain Viri ixodici encephalitidis № 205 for production of tick borne encephalitis vaccine; published Б.И. 05.04.80 № 13.)

The purification and inactivation steps are nearly identical for the two Russian TBE vaccines: Following a mouse brain passage, strain 205 is propagated in PCECs to produce the master seed, and following further passages in PCECsuspension cultures, the virus containing suspension is harvested, filtrated, and subsequently inactivated by formaldehyde. For concentration and further purification, ultrafiltration and gel-permeated chromatography on diol modified macroporous glasses are performed (Patent Russia №2203089, Krasilnikov I. at al., priority 28.06.2001).

The final version of this vaccine does not contain thiomersal. EnceVir® contains human albumin as stabilizer, but no preservatives, and is free from formaldehyde, gelatine, and bovine serum albumin. The vaccine is not lyophilized (is a liquid formulation) and adsorption to aluminium hydroxide is performed before filling.

The vaccine contains 2.0-2.5 µg of viral protein per dose. Antigen activity is determined serologically (required titre is $\geq 1:128$) by ELISA, produced by the Federal State Scientific-Industrial Company for Immunobiological Medicines of the Ministry of Health of the Russian Federation, MICROGEN, Tomsk, Russia.

EnceVir® is available in one formulation for adults. Half an adult dose is used to immunize children.

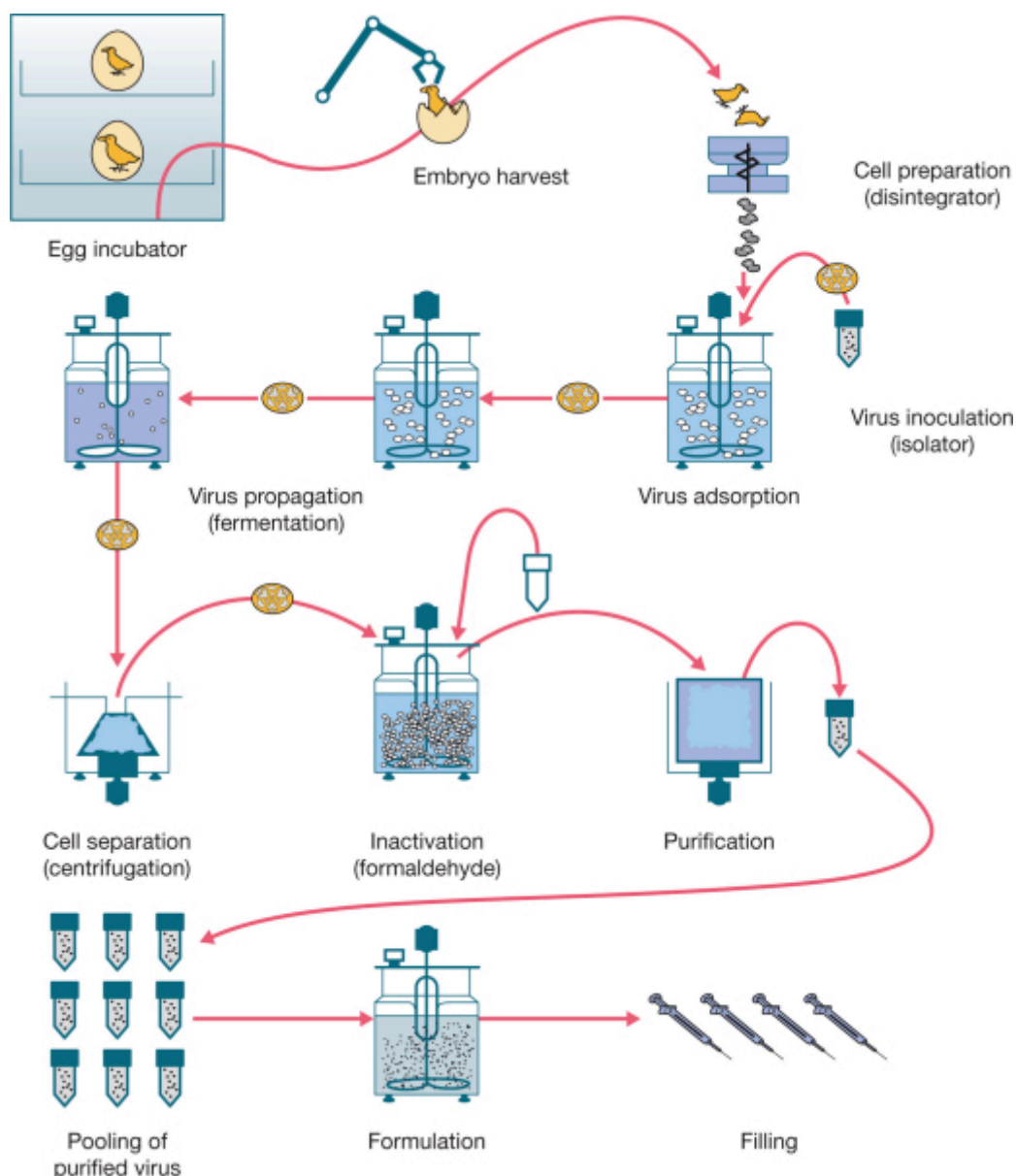
b. Manufacturing and quality control aspects

1. Encepur® and FSME-Immun®

Both Western vaccines are produced according to GMP rules and fulfil all quality criteria of modern vaccines including all controls, as required by EMA. Both vaccines have to be stored at 2-8°C and have a shelf life of 24 months. For details see (*Barrett et al., 2008*).

Figure 5 illustrates the main steps of TBE vaccine production and quality control.

Fig.5 (reprinted with permission from *Barrett et al., 2008*)



2. *EnceVir® and TBE-Moscow:*

Both vaccines are controlled by the National Regulatory Authorities, following WHO guidelines (*Vorob'eva et al., 2007; WHO TRS No 889, Annex 2, 1997*) and the manufacturers' own monographs.

Inactivation is controlled by biologic testing (intraperitoneal and intracerebral inoculation of 30 mice per lot of vaccine). The mice are euthanized 6-8 days after infection and a suspension of harvested brain material is injected intracerebrally to another group of 30 outbred mice (2nd passage). This procedure is repeated a third time (3rd passage). Mice are observed for 21 days.

Protective immunity of a new lot is compared to an industrial standard by challenging immunized mice with a lethal dose ($>300 \text{ LD}_{50}$) of TBEV strain Absettarov (European subtype).

Shelf life of the Russian vaccines is 3 years for TBE-Moscow vaccine and 2 years for EnceVir®. Although both vaccines require a cold chain they are stable for 2 days at 9-25°C.

c. Immunogenicity, antibody persistence, and effectiveness

Key points

1. There are no controlled trials on vaccine efficacy against clinical TBE; indirect evidence of protection is provided by trials using immunogenicity, mainly induction of neutralizing antibodies, as an endpoint.
2. Randomized, controlled trials as well as observational studies consistently show strong antibody induction (usually 85-100 % seroconversion rates) following a primary series of TBE vaccination. Similar results are obtained with all the 4 TBE vaccines.
3. Several observational studies which were followed up for ≥ 3 years showed persistence of neutralizing antibodies throughout this period
4. Although vaccine breakthrough infections occur, they are rare
5. Several observational studies testify to the high effectiveness of current TBE-vaccines in terms of TBE reduction in a population
6. The body of evidence is significantly larger for Western than for Russian vaccines

Encepur® and FSME-Immun®

Western TBE vaccines have been used for nearly 40 years and have proven to be highly efficacious in preventing TBE (*Heinz et al., 2007, Kunz, 2003*). For Encepur® and FSME-Immun® a considerable number of clinical phase I-III studies have been published. (For an overview, see Table 6). Both preparations are highly immunogenic. A number of serological test systems are currently used for antibody detection and presence of anti-TBEV antibody seem to be a consistent, reproducible, and a reliable marker of immunity against the disease (*Heinz et al., 2007, Holzmann, 2003, Holzmann et al., 1996*). However, no formal correlate of protection has been defined. Moreover, the manufacturers' data on immunogenicity are not directly comparable, since each company uses their own tests formats. No international reference reagents exist. Besides, head to head studies on TBE vaccines are rare and if available, they are rarely independently performed. However, the two vaccines show nearly identical immunogenicity results, both in children and adults, when vaccinated according to the conventional schedule (Table 5).

A recent Cochrane database review of the immunogenicity and safety of TBE vaccines (*Demicheli et al., 2009*), summarized data from 11 clinical trials - randomized or quasi randomized - controlled trials, mainly of Western vaccines. These trials included altogether

8.184 participants, 6586 adults and 1598 children. TBE-Moscow vaccine represented by one trial (*Pavlova et al., 1999*). The vaccines were compared with placebo, control vaccines, no intervention, or a different schedule or dose of the interventional vaccine. Immunogenicity was defined as seroconversion (by NT) following various immunization schedules. Demicheli et al conclude:

- i) Immunogenicity in children: With Encepur® (old formulation with polygeline), the seroconversion rates were between 97% and 99-100% (*Schondorf et al., 2007; Girgsdies and Rosenkranz, 1996*). For FSME-Immun the Cochrane review accepted only the trial on Ticovac® vaccine (formulation without human serum albumin), reported by Eder (*Eder and Kollaritsch, 2003*). This study revealed 95% seroconversion, after 2 or 3 vaccine doses; serological data following booster vaccination (9-12 months later) were not included in the review.
- ii) Immunogenicity in adults: With FSME-Immun, 96,6% seroconversion was observed for the licensed vaccine (*Ehrlich et al., 2003b*), for Encepur (*Schondorf et al., 2007*) seroconversion rates of 92% - 95% were obtained after 2 vaccinations. Corresponding data after booster doses were not evaluated.
- iii) With Encepur®, there were no significant differences of seroconversion rates following the different immunization schedules, but slightly higher rates were obtained with the rapid immunization schedule (i.e. 3 vaccinations on days 0-7-21). On the other hand, on day 300 the accelerated conventional schedule showed lower seroconversion rates.
- iv) The review states that neither study, irrespective of age, provided evidence showing that the involved vaccines caused severe adverse reactions, although local reactions were commonly observed.

The review by Demicheli *et al.* included studies on vaccines in use before 2001 and some more recent studies (*Pollabauer et al., 2010a; Pollabauer et al., 2010b*) were not included.

Table 6 summarizes all published data from controlled trials on currently licensed Western vaccines (actual formulation after 2001).

TBE Moscow vaccine® and EnceVir®

In the 1980ies, several clinical trials with the original formulation of TBE-Moscow vaccine resulted in licensing of this preparation in Russia. One of the first comparative evaluations of TBE-Moscow vaccine and FSME Immune was carried out both in an animal model and in humans (*Vorob'eva et al., 1996*). The studies conducted in mice showed no significant differences between the two vaccines in terms of immunogenicity and protective immunity when tested against different strains of TBEV. After two doses of TBE-Moscow or FSME Immune, HI- testing showed specific immune responses in 91% and 83%, respectively, of the

vaccinees. Also, in clinical trials, both TBE-Moscow and FSME Immune were found to be safe and well tolerated.

Pavlova et al., (1999) compared the immunogenicity of TBE-Moscow vaccine (current formulation) with the FSME-Immun (old formulation as given in Table 4) in children aged 7-17 years. As determined by at least four-fold increase in HI-antibody titer, 91.5% of the children immunized with the TBE-Moscow vaccine had seroconverted by day 28 after the second vaccination (schedule 0-4 months), as compared to 98.7% of vaccinees receiving the FSME-Imun® preparation. Mean HI- titers (fold increase of GMT after vaccination) was 1.75 (TBE-Moscow vaccine) versus 1.99 (FSME-Immun®). Based on these results, the TBE-Moscow vaccine with low content of cell proteins was recommended for vaccination of children and adolescents. Later, this formulation was also approved for immunization of adults.

Comparative clinical evaluation of the TBE-Moscow vaccine and EnceVir was carried out in 2001-2002 (*Gorbunov et al., 2002; Krasilnikov et al., 2002; Krasilnikov et al., 2004*). Four groups of adults (100 subjects per group) were vaccinated twice at intervals of 2 or 5 months. After two doses of the TBE-Moscow vaccine, HI-antibody titres $\geq 1:80$ against TBEV were detected in 84% and 93% of subjects, respectively. After two doses of EnceVir administered at the same intervals, antibody titres $\geq 1:80$ were demonstrated in 82% and 89%, respectively.

In 2003, the Russian National Regulatory Authority (Tarasevich State Institute for Standardization and Control of Medical Biological Products) completed a comparative, clinical evaluation of TBE-Moscow and EnceVir vaccines in 325 children stratified into three age groups: 3-6 years, 7-14 years, and 15-18 years. (*Pavlova et al, 2003b*). After two doses of TBE-Moscow vaccine ≥ 4 -fold increase of HI-antibody titres was demonstrated in 96%, 93% and 89%, respectively, of children of the involved age groups. The corresponding results with EnceVir were 84%, 97% and 92% , respectively.

Leonova and Pavlenko (2009) assessed the immunogenicity in adults of all four TBE vaccines using ELISA and a neutralization assay based on the P-73 strain of TBEV-Fe. All vaccines were found to be highly immunogenic and were subsequently recommended for large-scale vaccination in Russia. Successive administration of TBE Moscow vaccine and EnceVir using a combined vaccination protocol was also shown to induce high rates of seroconversion.

Although the Russian vaccines have demonstrated their protective qualities in the field, randomized, controlled studies on their efficacy/effectiveness have not been conducted.

Table 5: Summary of clinical studies with Encepur® (Novartis) and FSME-Immun® (Baxter). Clinical trials of Russian vaccines also included

Reference Study title (NCT-)Study number	Study period Country	Design	Description/Aim	No. of subjects planned (actual)	Summary	remarks used test system
Novartis						
Phase I, Phase II no published data available						
Phase III						
Clinical evaluation of a polygeline-free tick-borne encephalitis vaccine for adolescents and adults (Zent <i>et al.</i> , 2005, Zent <i>et al.</i> , 2003c) pooled data, 3 studies	Germany Czech Republic, Poland	0-7-21, controlled	immunogenicity and safety non-inferior to Encepur	3118 enrolled 12-76 years	high immunogenicity and good safety profile shown in adolescents and adults, non-inferiority to Encepur.	no exclusion of seronegative subjects, only NT-testing described
		Study A: controlled	immunogenicity non-inferior day 42 after 0-7-21	Adults	Non-inferiority day 42 SCR 100%	In- and exclusion-criteria not exactly given NT-K23
		Study B: observer-blind	immunogenicity prior booster day 21 during 0-7-21 safety non-inferior to Encepur	adolescents and adults	NT titers comparable in both groups SCR 100% titers present on day 21 in 100% non-inferiority in safety shown	
		Study C: No details given				
Kinetics of the immune response after primary immunization against tick-borne encephalitis (TBE) in adults using the rapid immunization schedule (Zent <i>et al.</i> , 2005, Zent <i>et al.</i> , 2003c)	Germany	open-label, multicenter, uncontrolled, prospective, follow up: booster 12-18 months after 0-7-21 with Encepur or Encepur adults (non-inferiority-study vs. Encepur)	Antibody persistence immunogenicity before and after booster: day 0, 21 safety	(222) healthy, 19-51 years	antibody levels sustained up to 12-18 months, high antibody response after booster (after prim. Immunization with either vaccine). safety of booster.	Enzygnost® Anti-TBE virus ELISA NT-K23
TBE booster immunization in adults--first experience with a new tick-borne encephalitis (TBE) vaccine, free of protein-derived		open-label, multicenter, uncontrolled, prospective follow-up	immune response 1 year after booster in preceding trial (0-7-21 with Encepur vs. Encepur adults and 12-18 months booster with	222 (190) 20-52 years	booster vaccination safe and effective up to 12 months, long lasting immune response suspected due to high antibody	

stabilizer (Zent <i>et al.</i> , 2005, Zent <i>et al.</i> , 2004)			Encepur adults)		titers	NT-K23
TBE booster immunization according to the rapid immunization schedule: are 3-year booster intervals really necessary? (Zent <i>et al.</i> , 2005, Zent <i>et al.</i> , 2003c)		open-label, multicenter, uncontrolled, prospective follow-up	immune response 1 and 2 years after booster in preceding trial (0-7-21 with Encepur vs. Encepur adults and 12-18 months booster with Encepur adults)	year 1: 191 year 2: 182 20-53 years	protective NT-titers sustained in 99% of subjects up to 2 years, kinetic curve suggests long persistence of antibodies; booster dose after 3 years seems not necessary.	no elderly persons NT-K23
Long-term immunity after vaccination against tick-borne encephalitis with Encepur using the rapid vaccination schedule. (Beran <i>et al.</i> , 2004, Zent <i>et al.</i> , 2005)	Czech Republic	open-label, singlecenter, uncontrolled, prospective follow-up	TBE-antibody levels before and 1 months after booster dose in subjects after 4 immunizations with Encepur (containing polygeline) at 0-7-21-month 15 in preceding trials	157 (148) ≥18 years	second booster more effective than first booster; high safety, long lasting immunity up to booster vaccination, strong immune response after basic immunization with Encepur adults	Enzygnost® Anti-TBE virus ELISA
Tick-borne encephalitis (TBE) vaccination: applying the most suitable vaccination schedule. (Schondorf <i>et al.</i> , 2007)	Czech Republic	controlled, open, randomized, single-center	Antibody titers day 0-21-42-180-300 (after booster day 300: titer day 321) Safety 4 subgroups: Immunogenicity on day 300 after 0-7-21 Immunogenicity on day 321 after 0-28-300 0-21-300 0-14-300	400 (398) ≥12 years 66 66 133 133	Highest antibody levels on day 42 after 0-28-300. Fast protection after 0-7-21 and highest antibody levels up to day 300	Enzygnost® Anti-TBE virus ELISA, NT-K23
Safety, immunogenicity and tolerability of a new pediatric tick-borne encephalitis (TBE) vaccine, free of protein-derived stabilizer. (Zent <i>et al.</i> , 2003a)	Germany Poland, Estonia	single blind, randomized, historically-controlled multicenter	sera at day 0-41 after 0-7-21 with Encepur children Lot-comparison of 3 batches Historical safety control: Encepur with 0.75 µg antigen (contained polygeline)	330 (404) (110/lot planned) stratification 1-5 years; 6-11 years	clinical lot-to-lot-consistency proven, immune response non-inferior to historical control, high safety	NT-K23

		controlled, randomized, , multicenter observer-blind at day 0, from day 7 on open-label	safety trial; vaccinations 0-7-21 non-inferiority (fever) versus FSME-Immun (no thiomersal)	2960 (3131) 1-11 years	non-inferior versus FSME-immun for fever $\geq 39^{\circ}$ after 1 st vaccination high safety and tolerability	only safety evaluation
Tick-borne encephalitis (TBE) vaccination in children: advantage of the rapid immunization schedule (i.e., days 0, 7, 21). (Schöndorf <i>et al.</i> , 2007)	Hungary	open-label, controlled, stratified-randomized, multicenter	3 groups basic immunization with Encepur children: <ul style="list-style-type: none"> • 0-7-21 • 0-28-300 • 0-21-300 Antibodies on day 0-42-180-300 (-321 after vaccination day 300u) safety	260 (294) 1-11 years 82 73 139 stratification 1-5 years; 6-11 years	Highest antibody levels on day 42 after 0-28-300 Highest antibody levels up to day 300 after 0-7-21	Enzygnost ® Anti-TBE virus ELISA, NT-K23
Phase IV						
Immunogenicity and safety of a booster vaccination against tick-borne encephalitis more than 3 years following the last immunisation. (Rendi-Wagner <i>et al.</i> , 2004a) M48P1	2002 Austria	uncontrolled, open-label, single-center	booster vaccination with encepur adults in subjects ≥ 3 years after documented basic immunization. Immunogenicity day 0, 21 safety	430 (426) ≥ 18 years stratification 18-49 years; ≥ 50 years;	Significant antibody increase after booster vaccination, independent from distance to last vaccination	Basic immunization not in a controlled trial /not under controlled conditions Enzygnost ® Anti-TBE virus ELISA, NT-K23
Antibody persistence following booster vaccination against tick-borne encephalitis: 3-year post-booster follow-up. (Rendi-Wagner <i>et al.</i> , 2007) M48P1E1	2005 Austria	prospective, uncontrolled, open-label, single-center extension of M48P1	immunogenicity 2 and 3 years after booster with Encepur adults in M48P1 antibody persistence (prospective)	430 Year 2: 195 Year 3: 240 stratification 18-49 years; ≥ 50 years;	persistence of antibodies in all subjects, boostable immune response after extended booster intervals	Enzygnost ® Anti-TBE virus ELISA NT-K23
Seroprotection 4 years following booster vaccination against tick-borne encephalitis. (Rendi-Wagner <i>et al.</i> , 2008) Booster vaccinations against tick-borne encephalitis: 6 years follow-up indicates long-term protection.	2006-2008 Austria	prospective, uncontrolled, open-label, single-center extension of M48P1	immunogenicity 4, 5 and 6 years after booster with Encepur adults in M48P1 antibody persistence and kinetics of antibodies (prospective)	430 Year 4: 198 Year 5: 225 Year 6: 195 stratification 18-59 years; ≥ 60 years;	persistence of antibodies in most vaccinees, kinetics of antibodies suggest long maintainance of antibody titers. Low rate of loss-responders in >60 years old subjects	Enzygnost ® Anti-TBE virus ELISA,

Multicenter Dose-Finding Study to Investigate the Safety and Immunogenicity of Two Vaccinations With FSME IMMUN NEW in Healthy Volunteers Aged 6 to 16 Years. NCT00161798 (Clinicaltrials.Gov, 2010)			all three vaccinations			Immunozyg ELISA PROGEN, NT-Neudörfl
Clinical evaluation to determine the appropriate paediatric formulation of a tick-borne encephalitis vaccine. (Pollabauer <i>et al.</i> , 2010a) Double-Blind, Randomized, Multicenter Dose-Finding Study to Investigate the Safety and Immunogenicity of Two Vaccinations With FSME IMMUN NEW in Healthy Volunteers Aged 1 to 6 Years. NCT00161772 (Clinicaltrials.Gov, 2010)	Mar02-Aug 02 Austria, Germany	randomized, dose comparison, double-blind, multicentric	safety and immunogenicity of doses 0.3; 0.6 or 1.2 µg of antigen randomization to one of the 3 doses; same dose administered for all three vaccinations	639 1-5 years	all subjects seroconverted after 1.2 µg; highly immunogenic and safe	no exclusion of children after other flavivirus-infections or vaccinations Immunozyg ELISA PROGEN, NT-Neudörfl
Phase III						
Comparison of immunogenicity and safety between two paediatric vaccines. (Pollabauer <i>et al.</i> , 2010b) Single-blind, Randomized, Phase 3B Study in Children Aged 1 - 11 Years to Investigate the Immunogenicity, Safety and Interchangeability of Two Tick-borne Encephalitis (Tbe) Vaccines Administered According to a Conventional Schedule NCT00840801 (Clinicaltrials.Gov, 2010)	Feb 09-Aug 10 Austria, Czech Republic	randomized, active control, single blind (subject) day 0-28 with FSME-Immun 0.25mL or Encepur children booster day 360 with FSME-Immun 0.25mL only	safety/efficacy Immunogenicity: NT-titers 28 days after vaccination 2. Interchangeability of 2 TBE-vaccines	303 1-11 years	non-inferiority of FSME immun 0.25 mL junior shown, immunological immune response higher after FSME Immun 0.25 mL, good safety profile Less adverse events after FSME-Immun 0.25 mL	No test systems using the K23 antigen analysis after vaccination 3 pending Immunozyg ELISA PROGEN, NT-Neudörfl; Enzygnost® Anti-TBE ELISA
Clinical evaluation to determine	Poland,	open label safety study	safety up to 35-42 days after dose	2417	excellent safety profile	Immunozyg ELISA

the appropriate paediatric formulation of a tick-borne encephalitis vaccine. (Pollabauer <i>et al.</i> , 2010a)	Austria, Germany		3; (subgroup of 400: immunogenicity)	1-15 years		PROGEN, NT Neudörfl
Safety and immunogenicity of the modified adult tick-borne encephalitis vaccine FSME-IMMUN: results of two large phase 3 clinical studies. (Loew-Baselli <i>et al.</i> , 2006) Single-Blind, Randomized, Multicenter Comparison of FSME IMMUN NEW and ENCEPUR: Safety and Tolerability of Two Vaccinations in Healthy Volunteers Aged 16 to 65 Years. NCT00161824 (Clinicaltrials.Gov, 2010)	Oct 01-Jan 02 Poland	controlled, randomized, multicenter, single-blind	FSME-Immun 0.5 mL or Encepur adults day 0 and 21-35 non-inferiority (fever after first vaccination) lot-consistency safety subset of 564: Immunogenicity	3966 (3927) 16-65 years	FSME Immun 0.5 mL non-inferior to Encepur adults lot-consistency demonstrated FSME-Immun 0.5 mL highly immunogenic and safe; Encepur adults boostable with FSME-Immun 0.5 mL	>65 years not included; healthy subjects only subjects tested positive for antibodies at baseline included in study No test systems using the K23 antigen
Safety and immunogenicity of the modified adult tick-borne encephalitis vaccine FSME-IMMUN: results of two large phase 3 clinical studies. (Loew-Baselli <i>et al.</i> , 2006) Open-Label, Multicenter, Follow-Up Phase III Study to Investigate the Safety of the Third Vaccination of FSME-IMMUN NEW in Volunteers Aged 16 to 66 Years NCT00161876 (Clinicaltrials.Gov, 2010)	May02-Aug02 Poland	non-randomized, uncontrolled, open-label, multicenter, follow-up	booster for subjects from preceding trial 180-day-booster with FSME-Immun 0.5 mL Safety subset of 564: Immunogenicity	3754 (3705)		Immunozyg ELISA PROGEN, NT-Neudörfl
Phase IV						
Seropersistence of tick-borne encephalitis antibodies, safety and booster response to FSME-IMMUN 0.5 ml in adults aged 18-67 years. (Loew-Baselli <i>et al.</i> ,	Jun04-Jul05 Poland	open label, single group, randomized, uncontrolled, follow-up	Immune response up to 3 years after dose 3 with FSME-IMMUN 0.5 mL Safety	340 (346) 18-67 years	high seropositivity rates 2-3 years after TBE-vaccination. boostable immune response, antibody titers significantly higher in subjects <50 years;	only subjects with sustained antibody levels included, no exclusion after contact with any

2009) Investigation of the Seropersistence of TBE Antibodies and the Booster Response to FSME-IMMUN 0.5 ml in Adults Aged 18 - 67 Years Follow-up to NCT00161876 (Clinicaltrials.Gov, 2010)					good safety profile	flavivirus-antigen, only subjects <67 years of age included Immunozyg ELISA PROGEN, NT-Neudörfl
Postmarketing surveillance						
Serological response to tick-borne encephalitis (TBE) vaccination in the elderly--results from an observational study. (Jilkova <i>et al.</i> , 2009)	2007 Czech republic	Non-interventional design, retrospective observation, monocentric	proportion of subjects with insufficient antibody response having history of 2 TBE vaccinations; antibody levels before and after vaccination 3 with FSME Immun 0.5 mL or Encepur adults	245 (185) >60 years	In persons >60 years 18% non-protective antibody levels after 2 vaccinations against TBE GMC and SCR higher after vaccination with FSME-Immun 0.5 mL importance of TBE-strains used in test systems	no information whether subjects with natural immunity participating; no samples saved for repeated analysis no randomization Immunozyg ELISA PROGEN in only 58 subjects: Enzygnost® Anti-TBE ELISA, Virology-In-house-ELISA 2 and NT-Neudörfl
Tolerability of modified tick-borne encephalitis vaccine FSME-IMMUN "NEW" in children: results of post-marketing surveillance. (Pavlova <i>et al.</i> , 2003)	Jan 01-Aug01 Austria	Observational, questionnaires only	FSME Immun 0.25mL as part of routine treatment, safety (fever) up to 3 days after vaccination	1922 (1899) 6 months-12 years	Mild fever in up to 20 % of subjects, good safety profile	Only subjects with completet follow-up included in analysis
Postmarketing, both preparations						
FSME monitoring: monitoring of adverse events of tick-borne-encephalitis vaccines by selected paediatricians and general practitioners. (Weinzettel <i>et al.</i> , 2007)	Feb 02-Jul 02 Austria	prospective, observational, multicenter questionnaires only	Monitoring of adverse events after administration of TBE vaccination in daily routine, questionnaires inquiring adverse events	25,907 all ages	Adverse events reported in 0.413%; total 107 reports, among them 69 concerned children up to 2 years. Reported adverse events in general mild	Total number of distributed dose per manufacturer was not recorded, therefore no comparison of relative frequency of

						adverse events.
Insufficient protection for healthy elderly adults by tetanus and TBE vaccines. (Hainz <i>et al.</i> , 2005)	Austria	Multicenter, retrospective	Blood draws in daily routine in persons vaccinated against TBE (primary immunization plus 2-5 booster injections at 3 years intervals) Evaluation of antibody levels in dependence of age and interval since last immunization	734 18-93 years	Age and interval since last booster immunization significantly influenced antibody levels against TBE. In 5-30% >60 years no protective antibody levels against TBE	No NT-testing performed On-site ELISA test
Federal state enterprise of Chumakov Institute of Poliomyelitis and Viral Encephalitides, Russia						
Comparative evaluation of safety immunogenicity of TBE-Moscow versus IMMUNE vaccine (Vorob'eva <i>et al.</i> , 1996)	1995, Russia	controlled, single-center	Safety and immunogenicity Vaccinated 0-2-months with 0,5ml Monitoring of adverse events after each administration of TBE vaccine in daily routine, questionnaires inquiring adverse events	100 18-23 years	Low reactogenicity Seroconversion by HI test after two doses in 91% of vaccines No significant differences in immunogenicity and protective activity induced by both preparations	Antibodies detected by HI and immunoenzyme assay HISTORIC PREPARATION!
Assessment of safety and immunogenicity of TBE-Moscow in comparison with FSME-Immune (Pavlova <i>et al.</i> , 1999)	1997-1998, Russia	Randomised, controlled, single center	Safety and immunogenicity Two doses at day 2 and 28 Serologic testing at 28 day after 2 dose	223 children 7-17 years	Low reactogenicity Antibody detected in 91% of vaccinated children Similar reactogenicity and immunogenicity of both vaccines	HI test
Assessment of safety and immunogenicity of TBE-Moscow versus EnceVir (Gorbunov <i>et al.</i> , 2002, Krasilnikov <i>et al.</i> , 2004)	2001, Russia	Controlled, single center	Safety and Immunoigenicity Vaccinated 0-2months or 0-5 months with 0,5ml per dose	200 adults, 50 per group	Moderate reactogenicity. Antibodies by HI test detected after two doses in 84% and 93% subjects vaccinated with intervals 2 and 5 months respectively	HI assay
Safety and immunogenicity of TBE-Moscow in paediatric trial (Report of the State Tarasevich Institute for Standardization and	2002-2003 Russia	Randomised, controlled, single centre	Vaccinated at 0 and 2 months Seven parameters of local and systemic reactogenicity tested Antibodies measured one month	Three age groups: 3-6 years, 8-14 years,	Moderated reactogenicity After two doses antibodies detected in 92%- 97% of children	HI and Immunoenzyme assay

Control of Medical Biological Products)			after second dose	and 15-18 years		
Assessment of humoral immunity of TBE-Moscow versus Encepur Adult, ESME-Immune and EnceVir against FE subtype of TBE virus (Leonova and Pavlenko, 2009)	2003-2007 Russia	Evaluation of immunogenicity, Prospective,	Comparative evaluation of immunogenicity. Antibody measured after 2-5 months and 2 years after primary vaccination with three doses of vaccines	47 adults vaccinated with TBE-Moscow	During first year and two years after priming antibody were shown by NT in 100% and 93% of vaccinated persons, respectively	NT and ELISA tests against strain P-73 of the Far Eastern subtype
Assessment of immunogenicity of TBE-Moscow after priming and booster (Stavitskaya <i>et al.</i> , 2004)	2002-2003 Russia	Controlled, single center	Boosting dose 12 months after priming with two doses. Antibody measured 12 months after second dose of priming schedule and one month after booster	43 children in three age groups: 3-6 years, 7-14 years, and 15-18 years	Seroconversion rate in all age groups after priming and boosting was 87%-90% and 100%, respectively.	HI test
Immunological and epidemiological effectiveness of mass immunisation programme (Romanenko <i>et al.</i> , 2007)	1996-2006 Russia	Mass immunization programme in Sverdlovsk Region, Russia	Comparative evaluation of four vaccines: TBE-Moscow, EnceVir, Encepur Adult, FSME-Immune	431 sera of subjects from 7 years old were tested	TBE-Moscow induced antibodies after second and third doses in 83% and 99% of vaccinees	Immunoenzyme assay
Microgen, Russia						
Assessment of safety and immunogenicity of EnceVir versus TBE-Moscow (Gorbunov <i>et al.</i> , 2002, Krasilnikov <i>et al.</i> , 2004)	2001, Russia	Controlled, single center	Safety and Immunoigenicity Vaccinated 0-2months or 0-5 months with 0,5ml per dose	200 adults, 50 per group	Moderate reactogenicity. Antibodies by HI test detected after two doses in 82% and 89% subjects vaccinated with intervals 2 and 5 months respectively	HI assay
Safety and immunogenicity of EnceVir in paediatric trial (Report of the State Tarasevich Institute for Standardization and Control of Medical Biological Products)	2202-2003 Russia	Randomised, controlled, single center	Vaccinated at 0 and 2 months Seven parameters of local and systemic reactogenicity tested Antibodies measured one month after second dose	325 children in three age groups:3-6 years, 7-14 years, and 15-18 years	Moderate reactogenicity Two doses seroconverted 84%-97% of vaccinees	HI assay

Assessment of humoral immunity of EnceVir versus TBE-Moscow, Encepur Adult and ESME-Immune against FE subtype virus (Leonova and Pavlenko, 2009)	2003-2007 Russia	Evaluation of immunogenicity, Prospective,	Comparative evaluation of immunogenicity. Antibody measured after 2-5 months and 2 years after primary vaccination with three doses of vaccine	47 adults vaccinated with TBE-Moscow	During first year and two years after priming antibody were shown by NT in 88% and 84% of vaccinated person, respectively	NT and ELISA tests against strain P-73 of the Far Eastern subtype
Assessment of immunogenicity of EnceVir after priming and booster (Stavitskaya <i>et al.</i> , 2004)	2002 - 2003 Russia	Controlled, single center	Boosting dose 12 months after priming with two doses. Antibody measured 12 months after second dose of priming schedule and one month after boosting	88 children in three age groups: 3-6 years, 7-14 years, and 15-18 years	Serocoverion rate in all age groups after priming and boosting was 78% - 89% and 100%, respectively	HI test
Immunological and epidemiological effectiveness of mass immunisation programme (Romanenko <i>et al.</i> , 2007)	1996-2006 Russia	Mass immunization programme in Sverdlovsk Region, Russia	Comparative evaluation of four vaccines: TBE-Moscow, EnceVir, Encepur Adult, FSME-Immune	431 sera of subjects from 7 years old were tested	EnceVir induced antibodies after second and third doses in 83% and 99% of vaccinees	Immunoenzyme assay

d. Schedules for basic immunization

Key points (d. Schedules for basic immunization and e. Booster schedules)

1. Western TBE manufacturers recommend 3 doses for a complete primary course of immunization. Conventional dose intervals are 1-3 months between doses one and two, and 9-12 months between doses two and three
2. With Russian vaccines, standard primary immunization schedule consists of two doses given at an interval of 1-7 months. A third dose (first booster) is recommended 12 months after the second dose
3. "Rapid" or "accelerated" schedules for emergency situations are based mainly on reduced intervals between the first 2 doses
4. All manufacturers recommend booster immunizations 3 years after completion of the primary series/third dose. With Western vaccines, subsequent boosters are given at intervals of 5 years (3 year intervals for individuals aged >60 years). Russian manufacturers recommend boosters at 3-year intervals, throughout

Table 6: Immunization schedules for TBE- vaccines according to manufacturers recommendations.

schedule	Encepur®					
	Primary immunization (days)				Boosters (years)	
	First	Second	Third	Fourth	First booster	Subsequent boosters
conventional	0	28-90	270-365	-	3	5(3*)
rapid	0	7	21	365-540	5(3*)	5(3*)
	FSME-Immun®					
	First	Second	Third	Fourth	First booster	Subsequent boosters
Conventional	0	28-90	270-365	-	3	5(3*)
accelerated	0	14	150-365	-	3	5(3*)
	TBE vaccine Moscow					
	First	Second	Third	Fourth	First booster	Subsequent boosters
conventional	0	30-210	-	-	1	3
	EnceVir®					
	First	Second	Third	Fourth	First booster	Subsequent boosters
conventional	0	150-210	510-570	-	3	3
rapid	0	21-35**	42-70**	150-365	3	3

*) Boosting every 5 years, but 3 years only for persons > 60years of age

**) double dose of 1,0ml

Encepur® and FSME-Immun®

3 licensed schedules for primary immunization have been described (see Table 6):

- “conventional immunization schedule”:
immunizations on day 0, after 1-3 months, and after 9-12 months
- “Rapid immunization schedules”:
For Encepur® a rapid immunization schedule is licenced with 3 vaccinations on days 0-7-21 and a first booster after 12-18 months ;
- For FSME-Immun® the rapid immunization (“modified or accelerated conventional schedule”) consists of vaccinations on day 0 and 14 and an (early) third vaccination after 5-12 months (for the adult formulation).

As shown in Table 5, both Western vaccines show excellent immune responses after the third immunization of a conventional schedule. All studies, irrespective of the basic schedule, showed seroconversion rates close to 100% both in children and adults. However, there are no studies on the primary immune response in persons above 60 years of age.

The rapid and the conventional schedules of Encepur® induce similar responses (*Zent et al., 2005; Wittermann et al., 2009b; Schondorf et al., 2007*). Seroconversion appeared as early as 2 weeks after the second vaccine dose in 98% of those who were immunized according to the conventional schedule, and in 90% of those who received the vaccine according to the rapid schedule. This confirms the capacity of the rapid schedule to induce rapid protection (*Schondorf et al., 2007*), which may be advantageous for travel- related TBE vaccinations (*Zent et al., 2005*).

The accelerated schedule of FSME-Immun® induces an earlier antibody response than achieved by the conventional schedule, but also a faster decline of antibodies by day 300. Thus, the accelerated schedule may be associated with an earlier loss of protection (*Schondorf et al., 2007*).

Overall, antibody decline is considerably faster after the third dose of the conventional schedule than after the fourth and subsequent doses, i.e. first and subsequent boosters (*Rendi-Wagner et al., 2004b*). This observation led to the current recommendation that the first booster should be administered 36 months after completion of the primary series and subsequent boosters at intervals of 60 months.

All basic immunization schedules are similar in terms of anamnestic responses and antibody titers (see below).

EnceVir and TBE vaccine Moscow

Both vaccines use the same schedules:

- Conventional schedule: the second vaccine dose is given 5-7 months after the first immunization, and a third injection 6-12 months thereafter completes the primary series. Revaccinations (boosters) are given at intervals of three years.
- Alternative schedule: The first vaccination is followed by a double dose after 3-5 weeks and a third vaccination – also with a double dose – is given 3-5 weeks after the

second dose. A fourth dose is administered after 6-12 months and subsequent revaccination every 3 years.

In Russia, published evaluations comparing different schedules of immunization against TBE are limited. *Stavitskaya et al., (2004)* evaluated the immunogenicity of the Russian vaccines in a paediatric trial that included 325 children/adolescents 3-18 years old; all vaccinated twice with a two months' interval between injections. Twelve months after this primary vaccination, 131 children were revaccinated with one dose of the same vaccines. Antibody titres were measured by a HI-test. Of children aged 3-6 years who received EnceVir, 100% had seroconverted (mean titer 1,96) by day 28 after the second dose (schedule 0-60 days). Twelve months after the second dose, antibody (mean titer 1.32) was still detected in 72% of the children. The third dose induced a 100 % serological response in this group, with a mean titer of 2,39. Of the children aged 3-6 years who received two doses of the TBE-Moscow vaccine, 100% had seroconverted (mean titre 2,27) by day 28; 12 months later, antibodies (mean titer 1,49) were still demonstrated in 87% of the vaccinees. The third dose of TBE Moscow vaccine induced a serological response (mean titer 2,88) in all children. Similar dynamics of immune responses were observed in age groups 7-14 years and 15-18 years. These findings were seen as a confirmation of the high immunogenicity both of the EnceVir and TBE-Moscow vaccines.

With all the 4 vaccines (TBE-Moscow vaccine, EnceVir, FSME-Immun, and Encepur) used during the mass immunization programme against TBE conducted in the Sverdlovsk Region, Russia, increased seroconversion rates were observed following the second dose of the primary vaccination series (*Romanenko et al (2007)*). Thus, as demonstrated by ELISA, the first and second doses of TBE-Moscow vaccine induced seroconversion in 59% and 83%, respectively, of the vaccinees, whereas the corresponding figures with EnceVir were 74% and 85%.

Irregular schedules

Information is scanty on the efficacy and effectiveness of TBE vaccines when the primary vaccination series has been interrupted or the intervals between the scheduled doses have been grossly extended. However, a recent study by Schosser (*Schosser, 2009*) concluded that in 94% of vaccinated subjects even the first TBE immunization mounts a long lasting immune memory. This finding suggests that extended intervals between the first two or three vaccinations are not critical for the success of subsequent immunizations. This conclusion is supported also by the study of Heinz *et al., (2007)*, who showed that even under assumption of a worst case scenario the field effectiveness also for irregularly vaccinated subjects was around 95%. Still, in irregularly vaccinated individuals, the risk of TBE following exposure to TBEV was calculated at 3-8 times higher than in regularly vaccinated subjects.

It is accepted that Encepur® and FSME-Immun® can be used interchangeably (see Table 6) (*Broker and Schondorf, 2006*). However, when possible, it is recommended to use the same TBE vaccine throughout the basic immunization series.

For the Russian vaccines no information on irregular vaccination schedules and interchangeability of vaccines are available.

e. Booster schedules and persistence of immunity

In this review, we define the first booster (i.e. dose 4) within a conventional, or accelerated conventional, TBE immunization schedule as the vaccination which is scheduled 3 years after completion of the primary 3-dose series¹ (see Table 6).

Encepur® and FSME-Immun®

For the rapid immunization schedule of Encepur®, the first booster is scheduled already after 12-18 months. For the conventional schedule, after the first boost (year 3), boosting intervals are extended to five years, except for persons aged ≥ 60 years, who should receive boosters at intervals of 3 years.

With regard to immunogenicity results after this first booster, both Western vaccines showed excellent results with serological response rates near 100% and high GMTs with the respective NTs (Table 6).

With both Western vaccines, boosting responses are relatively good also in the elderly (*Rendi-Wagner et al., 2004a; Loew-Baselli et al., 2009*) although undoubtedly, in those aged ≥ 60 years the immune response is weaker and probably lasting for a shorter time (*Paulke-Korinek et al., 2009; Rendi-Wagner et al., 2007; Loew-Baselli et al., 2009*). However, there is no clinical evidence for a substantially higher failure rate of TBE vaccination in the elderly population (*Hainz et al., 2005; Heinz et al., 2007; Weinberger et al., 2007*). Although vaccine breakthroughs occur more often in older persons (*Andersson et al., Heinz et al., 2007*), the overall breakthrough rate is low.

The immunological response to a booster is not significantly influenced by the length of the interval to the previous dose of the vaccine (booster or primary series); moreover, the increase in antibody titer is reciprocal to the titer before the booster (*Rendi-Wagner et al., 2004a*).

The boosting recommendations that are listed in the package leaflet for Encepur® and for FSME-immun® are slightly different due to minor differences of the recommended basic schedules (see Table 7).

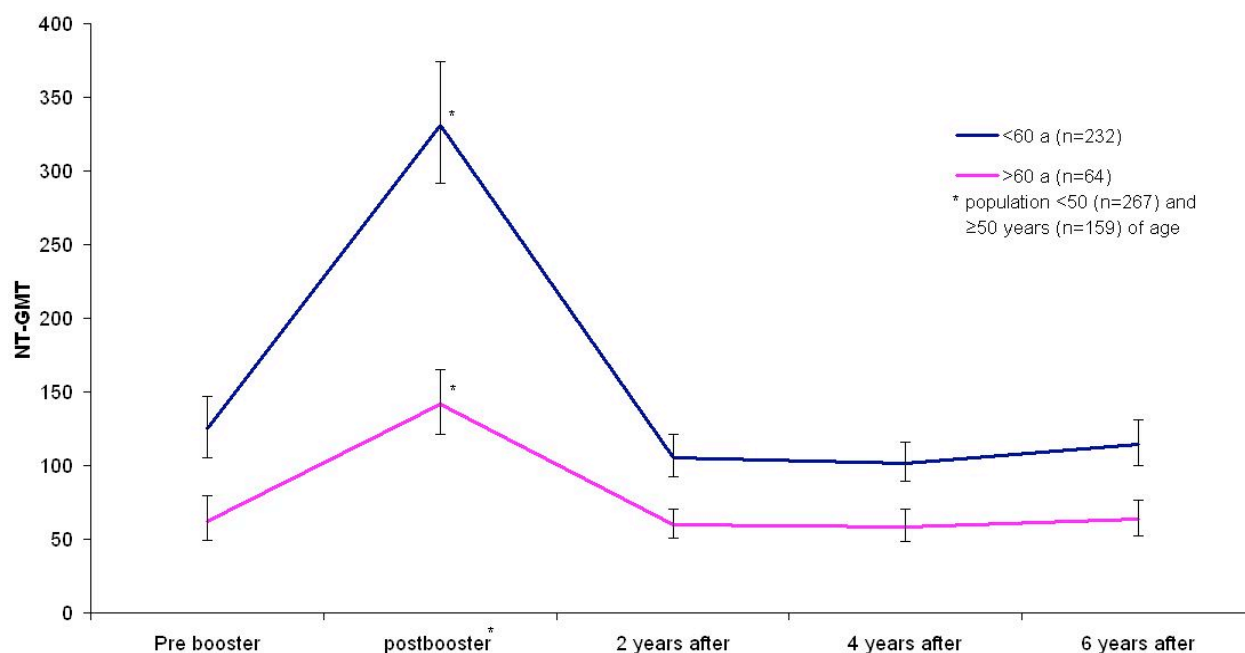
Since the introduction of TBE vaccines, the persistence of immunity following boosters has been a matter of debate. A few recent studies suggest that protection may last for much longer time than generally predicted. In a cross sectional study of Encepur (*Rendi-Wagner et al., 2004b*) the annual antibody decline was found to be only about 6.7% after the first (or a subsequent) booster, while this decline reached 18% in subjects who had received basic

¹The manufacturers of TBE Moscow vaccine define the first 2 doses as the primary series, and dose 3, which is due 6-12 months after the second dose, as the first booster.

immunization only (i.e. 3 vaccine doses; see Table 7) . Similar results were shown recently for FSME-Immun (Loew-Baselli et al., 2009).

Longitudinal studies in pre-boostered subjects showed that after a booster, the yearly antibody decline did not exceed 8% per year (Paulke-Korinek et al., 2009; Rendi-Wagner, 2008; Rendi-Wagner et al., 2007). A similar decline irrespective of age was observed in all studies; nevertheless older persons (>60 years of age) faced the risk of becoming earlier seronegative because of the lower antibody titer achieved immediately after the booster (Fig.6) (Loew-Baselli et al., 2009; Paulke-Korinek et al., 2009; Rendi-Wagner et al., 2004b; Rendi-Wagner et al., 2007; Weinberger et al., 2007). Other data provide evidence that in more than 90% of vaccinees, protection after TBE booster vaccinations exceeds the time recommended as booster intervals, and that in most vaccinees antibody levels remain stable for at least 8 years (Paulke-Korinek et al, to be published) (see Fig 6). It should be noted, however, that NTs are semiquantitative only, and vary with laboratory settings. This complicates precise calculations of the annual rates of TBEV-specific antibody decline. Long-term observation indicate that even 8 years after the last dose of TBE vaccine, GMT- NT titers in vaccinated elderly subjects are at 60 – and in younger persones at 80 (Fig.6).

Fig.6: Antibody kinetics after TBE booster vaccination (adapted from Paulke-Korinek et al., 2009; Rendi-Wagner et al., 2004a; Rendi-Wagner et al., 2007).



However, as antibody levels in persons > 60 years are 2-fold lower overall, this group faces a higher risk of becoming early seronegative. Old persons who were young when first immunized will respond to boosting similar to young people, while those who started their primary course at the age of ≥60 years will a priori develop lower antibody titers, and lower ability to respond to recall antigens (Weinberger et al., 2007).

Referring to the recently documented long persistence of antibodies against TBEV, boosting intervals for TBE vaccinations have been extended to ten years, at least in Switzerland, (*Bundesgesundheitsamt, 2008*). Proof of concept for this recommendation is under way.

EnceVir® and TBE vaccine Moscow®

Boosting is currently recommended every 3 years (Table 7). Following primary TBE-immunizations and boosting of 431 individuals during the 1996-2006 mass immunization programme in the Sverdlovsk region, Russia, *Romanenko et al., (2007)* reported on the immune responses to the involved four TBE vaccines. No information on intervals between vaccine administrations is provided in this publication. An ELISA was used to measure anti-TBE antibodies. After two primary vaccine administrations and after a third (“booster”) dose, the TBE-Moscow vaccine induced seroconversion in 83% and 99%, respectively. In the EnceVir- vaccinated group, the corresponding figures were 84% and 98%. (The corresponding results were 92% and 96% with FSME-Immune vaccine and 40% and 95% with Encepur).

Leonova and Pavlenko (2009) assessed persistence of antibodies in adults vaccinated with either TBE-Moscow vaccine, EnceVir, FSME-Immune Inject or Encepur. During the first year and two years after primary vaccination (three doses), virus-neutralizing antibody (against a TBEV-Fe strain) were detected in i) 100% and 94% of TBE-Moscow vaccinees; ii) 88% and 84% of subjects receiving EnceVir; iii) 88% and 78% of FSME-Immune Inject vaccinees; and in iv) 100% and 100% of volunteers immunized with Encepur. All four vaccines were subsequently recommended for use in mass immunization in the Far Eastern region of Russia where the most pathogenic strains of TBEV are circulating. Surveillance after 3 primary doses of EnceVir vaccine demonstrated persistence of high levels of TBE-specific antibody for at least 3 years (*Il'ichenko et al, 2009*).

With the Russian vaccines, there are no details on the immunogenicity achieved in elderly persons and no details on long-term persistence of immunity.

f. Safety and reactogenicity

Key points

1. All randomised, controlled trials (RCTs) on vaccine safety are based on Western vaccines. For Russian vaccines, only two observational studies are available.
2. RCTs as well as observational studies consistently describe current TBE vaccines as moderately reactogenic (redness/pain at injection site in up to 45%, fever in up to 5-6% of vaccinees), but no severe adverse events has been causally linked to TBE vaccines
3. Large post-market surveillance in Austria did not identify any serious vaccine-associated adverse events

Encepur® and FSME-Immun®

Safety data for a total of more than 10.000 participants were obtained as part of the clinical phase II and III studies (Table 6). Severity, incidence and nature of the adverse events were similar with the different preparations. Table 8 shows reactogenicity of Encepur® and FSME-Immun® according to the WHO classification criteria.

Table 7. Reactogenicity of Encepur® and FSME-Immun® (source: SMPC)

Probability	≥1/10	≥1/100 <1/10	≥1/1000 <1/100	≥1/10.000 <1/1000	Not known
FSME-Immun® First vacc.: n= 3512 Second vacc.: n= 3477 Third vacc.: n=3277	Local reaction at injection site: Redness, swelling, induration	Headache, nausea, Myalgia, arthralgia, Malaise, fatigue	Lymphadenopathy, Vertigo, Vomiting Fever (only exceptionally > 39°C)	Acute allergic reactions; Somnolence, Diarrhea, abdominal pain;	Aggravation of autoimmune disease; visual impairment, photophobia, Meningism, epilepsy, encephalitis, neuritis; tachycardia; Urticaria, pruritus, exanthema; Flu like symptoms, weakness, oedema
Encepur® (pooled data from clinical studies and postmarketing surveillance)	Transient pain at injection site; general malaise, myalgia, headache	Redness, swelling at injection site; Flu-like symptoms; Nausea, Arthralgia	Arthralgia and myalgia (neck);	Granuloma at injection site; Lymphadenopathy, Neuritis-like symptoms; Diarrhea; Systemic allergic reactions like urticaria, dyspnoe, bronchospasm, hypotension	Extremely rare: Guillain-Barre syndrome

In 2002, an independent postmarketing study conducted by the Institute for Vaccine Safety of the Austrian Green Cross monitored 25.905 vaccinations (Encepur® and FSME-Immun®)

that had been carried out by general Austrian practitioners (*Weinzettel et al., 2007*). This survey identified 107 adverse events, corresponding to an adverse event percentage of 0,413% among all vaccinees. Of these events, 69 (64,5%) occurred in siblings and toddlers up to two years of age; 31% were local reactions and 9% suffered from pain at the injection site. In 63 cases fever was reported, 45 of these patients had mild fever (38-39°C), 15 moderate (39-40°C) and 3 high fever (> 40°C). Of these 107 adverse events, 52 (48,6%) were registered after the first vaccination; in children 75,8% occurred after the first vaccination (*Weinzettel et al., 2007*).

Postmarketing pharmacovigilance data collected for many years by the vaccine manufacturers provide evidence for an excellent tolerability of both Western vaccines. Thus, no adverse events leading to a change of the overall risk-benefit profile of Encepur® and FSME-Immun® were reported during more than 8 years of surveillance (FSME-Immun, Table 9a; Encepur, Table 9b). Although these pharmacovigilance data are not derived from controlled trials, they reflect accumulated practical experience of TBE vaccination in daily practice.

Concluding from clinical studies and pharmacovigilance, the Western TBE vaccines are safe and well tolerated. In terms of safety and reactogenicity there are no obvious differences between the two Western vaccines.

Table 8a Postmarketing surveillance data for FSME-Immun® (Baxter, data on file)

Global Adverse Reaction Incidences of FSME-Immun				
Vaccine	Doses	Number of reports (incidence/100.000)		
		Serious	Non-serious	total
FSME-IMMUN*	41 973 932	658 (1.57)	1764 (4.20)	2422 (5.77)

*) currently licensed formulation; period of 29 Jan 2001 until 30 Nov 2009, only spontaneous, literature and regulatory reports

Table 8b Postmarketing surveillance for Encepur (Novartis, data on file)

Global Adverse Reaction Incidences of Encepur®*				
Vaccine	Doses	Number of reports (incidence/100.000)		
		Serious	Non-serious	total
Encepur adults	21,005.778	2,0	5,5	7,4
Encepur children	9,149.333	1,7	3,5	5,2
Both formulations	30,155.111	1,9	5,9	7,7

* for period 1 January 2002 - 31December 2009

EnceVir and TBE vaccine Moscow

In the years 2002-2003, the Tarasevich State Institute for Standardization and Control of Medical Biological Products assessed the safety of TBE-Moscow and EnceVir following

immunization of 400 adults (data not published). Based on analyses of eight different parameters of systemic and local reactogenicity both vaccines were found to be low reactogenic.

Pavlova et al, (2003b) studied the safety of TBE-Moscow vaccine and EnceVir in 325 children/adolescents of the age groups 3-6 years, 7-14 years, and 15-18 years. Local reactogenicity (pain, redness, and enlargement of lymph nodes) and systemic reactogenicity (fever, malaise, headache, nausea, arthralgia) were assessed 4-5 days after each vaccination. No severe adverse events were recorded and there were no statistically significant differences in frequency of systemic and local reactions between the two vaccines.

The safety of TBE-Moscow and EnceVir has been confirmed in several other studies, but little detailed information has been published (*Pavlova et al., 1999; Vorob'eva et al., 2007; Krasilnikov et al., 2002*). However, post-marketing surveillance of EnceVir and TBE-Moscow vaccines has not revealed any severe adverse events (*Borodina, 2004, Il'chenko et al., 2009*). The Russian National Regulatory Authority has assessed TBE-Moscow and EnceVire vaccines and concluded that they are both safe and well tolerated. In an unblinded study (Table 9c) comparing the TBE Moscow vaccine with FSME-Immun (old formulation) in children *Pavlova et al., (1999)* concluded that reactogenicity does not differ significantly between these two Russian vaccines.

Table 8c Adverse reactions: Comparing the TBE Moscow vaccine with FSME-Immun (*Pavlova et al., (1999)*)

All temperature reactions		Russian vaccine						Austrian vaccine					
		All children inoculated		Age 7-10		Age 11-17		All children inoculated		Age 7-10		Age 11-17	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Normal temperature		91	79.8 ±3.7	23	92.0 ±5.4	68	76.4± 4.1	103	96.3 ±1.8	26	100	77	95.1 ±2.4
Slight reaction (37.1- 37.5° C)	Total	16	14.0 ±3.2	2	8.0 ±5.4	14	15.7 ±3.8	4	3.7 ±1.8	-		4	4.9 ±2.4
	After 14- 16 hrs	4	3.5 ±1.7	2	8.0 ±5.4	2	2.2 ±1.1			-		-	
	After 24 hrs	12	10.5 ±2.9	-		12	13.5 ±3.6	4	3.7 ±1.8	-		4	4.9 ±2.4
Moderate reaction (37.6- 38.5° C)	Total	6	5.2 ±2.1	-		6	6.8 ±2.7	-		-		-	
	After 14- 16 hrs	3	2.6 ±1.5	-		3	3.4 ±1.9						
	After 24 hrs	3	2.6 ±1.5	-		3	3.4 ±1.9						
Severe reaction (38.6° C)		1	0.9 ±0.8	-		1	1.1 ±1.1	-		-		-	
No. of children observed		114		25		89		107		26		81	

IV Outcomes of immunization

Key points (a Field effectiveness of vaccination b. Impact of vaccination)

1. During 30 years, the Austrian immunization program reduced the yearly incidence of TBE to 10% of prevaccination levels
2. In regularly vaccinated individuals in Austria, field effectiveness of Western vaccines was calculated at 99.3% - 98.7%; effectiveness of the first two injections was 98,7% -100%
3. After mass immunization in Sverdlovsk 2000-2006, field effectiveness estimates for TBE Moscow vaccine was 62,3% - 88,6%.
4. In Krasnoyarsk 1999-2003, following large-scale immunization using mainly TBE-Moscow vaccine, the incidence of TBE was 20 times lower in vaccinated as compared to non-vaccinated groups

g. Field effectiveness of vaccination

Given the overall low incidence of the disease, no randomized controlled trials against clinical endpoints have been conducted. Also, as the vaccination coverage in most endemic countries is too low to allow firm conclusions concerning its impact on TBE morbidity, studies on the field effectiveness of TBE vaccines are rare. For these reasons, assessments of the efficacy of TBE vaccines are based on serologic markers of protection, primarily the NT-test (*Holzmann et al., 1996*).

However, within a period of 20 years, nearly 90% of the Austrian population were reached by at least one dose of TBE vaccine, and during this period, the number of TBE cases in Austria decreased steadily (*Heinz and Kunz, 2004; Kunz, 2003; Kunz and Heinz, 2003*).

The field effectiveness of TBE vaccination was determined in a study by Heinz (*Heinz et al., 2007*). This study is considered representative for both Western vaccines, although FSME-Immun® is the clear market leader in Austria. In this study, the incidence of laboratory-confirmed, hospitalized TBE cases with neurological symptoms was analyzed by age and TBE-vaccination status for the period 2000-2006. The vaccination status was established annually by inquiries to 8.500-10.000 representative individuals. The term “regularly vaccinated” was defined as those who had received a complete series of three vaccinations, with or without additional boosters, at regular intervals. “Irregularly vaccinated” were those, who fell outside the recommended vaccination schedule. Best- and worst case scenarios were calculated by adding persons with unclear vaccination histories to the regularly or irregularly vaccinated groups, or by excluding them from the calculation. During the observation period, 494 cases of TBE were registered in Austria, and this number served as basis for the calculation of field effectiveness. For details see (*Heinz et al., 2007*).

Table 9. Field effectiveness of TBE vaccination in Austria. Adapted after *Heinz et al., 2007*

	Unvaccinated (incidence/100.000)	Regularly vaccinated (incidence/ 100.000)	Field effectiveness 95% CI	Irregularly vaccinated (incidence/ 100.00)	Field effectiveness (95%CI)
Best case Scenario*)	5.922	0,039	99,3 (98,92-99,56)	0,212	96,4 (95,1-97,34)
Worst case Scenario**)	5.922	0,079	98,7 97,98-98,67		94,6 (92,7-95,87)

*) best case scenario: TBE cases with unknown or undefined vaccination status were not considered to belong to the “regularly” vaccinated group.

**) worst case scenario: TBE cases with unknown or undefined vaccination status were considered to belong to the “regularly” vaccinated group.

Moreover, this study allowed field effectiveness of the first two injections of the basic immunization to be estimated at 98,7% and 100% for the worst case and best case scenarios, respectively (*Heinz et al., 2007*).

For the Russian vaccines data on clinical efficacy and effectiveness are limited. *Romanenko et al., (2007)* compared the number of TBE cases in vaccinated and non-vaccinated groups after the mass immunization programme in the Sverdlovsk Region (see Table 11). This study was based on all the four currently licensed TBE vaccines, represented, however, by different shares of the total vaccination coverage: Moscow-vaccine (80%), EnceVir (6%), FSME-Immun (12%) and Encepur (2%). An effectiveness estimates was possible for TBE Moscow vaccine only, and found to be within a range of 62,3 to 88,6%.

Table 10: Comparative assessment of TBE morbidity in vaccinated and non-vaccinated cohorts, 2000-2006 (*Romanenko et al., 2007*)

Year	TBE morbidity			Morbidity in inoculated persons per 100 000	Morbidity in noninoculated persons per 100 000	Epidemiological efficacy (%)
	Total	Inoculated	Noninoculated			
2000	453	124 (27)	329 (73)	6.5	17.4	62.3
2001	426	135 (32)	291 (68)	6.9	15.9	63.1
2002	418	96 (23)	322 (77)	4.5	18.0	76.7
2003	362	67 (18)	295 (82)	3.1	17.7	83.8
2004	315	60 (19)	255 (81)	2.6	16.2	84.0
2005	448	95 (21)	353 (79)	3.4	22.6	85.0
2006	228	45 (19)	183 (80)	1.5	13.0	88.6

Note: figures in parentheses indicate %.

Vaccine breakthroughs are rare, but they do occur. Descriptions of breakthrough infections are available for Western TBE vaccines only. *Stiasny et al., 2009*, reported on 25 breakthroughs in Austria during the years 2002-2008; 8 of these occurred in fully vaccinated individuals. *Andersson et al., (2010)*, described 27 break-through cases in Sweden during the years 2000- 2008, of whom 21 had received a full course of TBE vaccination. Both authors state that patients with a breakthrough infection have delayed and low-titered IgM- responses and a very rapid and high-titered IgG- response. If in such patients only one serum sample is taken, a confusion with postvaccinal immunity may result, since IgM appears late (*Andersson et al., 2010, Stiasny et al., 2009*). The antibody profile of vaccine break-throughs was found to be characteristic of an anamnestic immune response, indicating that in those cases the immunological priming and memory had not been sufficient, or fast enough, to prevent disease. More than 70% of vaccine breakthroughs occurred in persons older than 50 years. This emphasizes the need for shorter booster intervals and/or serologic immunity controls in elderly persons, particularly those, who are at high risk of TBEV exposure and/or have an underlying immunocompromising disease (*Andersson et al., 2010; Hainz et al., 2005; Loew-Baselli et al., 2009; Paulke-Korinek et al., 2009*).

Cross-protection against TBE by different subtypes of TBEV

Although the genetic homology between TBEV strains strongly suggests that vaccination with one subtype will induce cross protection against all subtypes, clinical correlates for this assumption are limited so far. A recent study in mice using FSME Immun® post immunization sera showed identical neutralization titers against the European, Siberian, and Far Eastern TBE subtypes suggesting protection against all naturally circulating variants of TBEV (*Orlinger et al., 2011*)

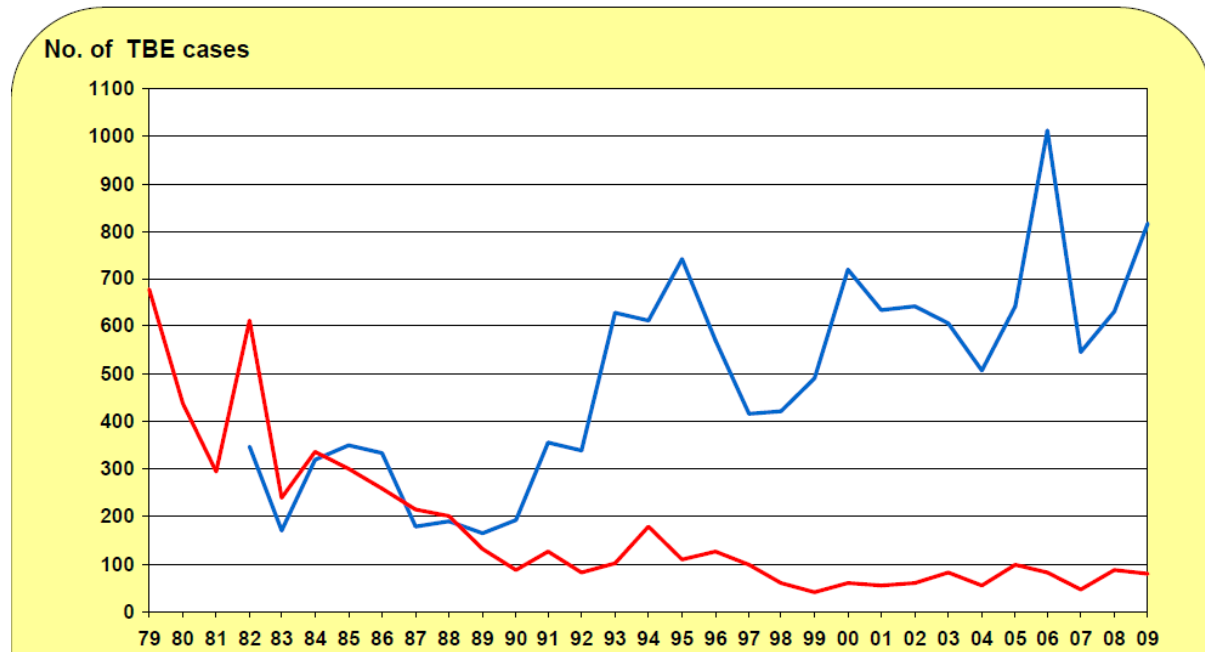
h. Impact of vaccination

TBE case-reporting tends to be unreliable in regions where the disease is endemic (*Suss, 2008, Suss, 2010*). Besides, in order to prove the effectiveness of a vaccination programme a large proportion of the population at risk must be vaccinated, long term quality reporting of TBE cases is required, and monitoring of fluctuations in TBEV-prevalence of the tick-population ensured. Unfortunately, in Europe almost no country fulfils these criteria.

Comprehensive data are available from Austria, where mass vaccination campaign against TBE were put in place more than 20 years ago and vaccination coverage has exceeded 85% for more than a decade, while the endemic areas remain largely unchanged (*Heinz, 2008; Heinz and Kunz, 2004; Kunz, 2003; Suss, 2008; Suss, 2010*). Therefore, in Austria the impact of vaccination can be described relatively exactly. In addition, the Czech Republik, neighbouring Austria and facing similar endemicity and ecological conditions, but with only about 15% TBE vaccination coverage, may serve as “control”.

Fig 7. Comparison of TBE cases during the years 1979-2009 in Austria (red) and the Czech republic (blue). (Courtesy: FX.Heinz, Institute of Virology, Medical University of Vienna)

TBE in Austria and Czech Republic 1979-2009



Heinz *et al.*, (2007) calculated that during the period 2000-2006, about 2.800 TBE cases, including 20 deaths, were prevented by the Austrian vaccination campaign, assuming that the incidence of TBE would not have changed significantly over the years. In fact, endemic areas were spreading during this period, particularly in Western part of Austria (Holzmann *et al.*, 2009).

During its 30 years of operation the national immunization program in Austria has reduced the yearly incidence of TBE to an average of 65 -75 cases per year , which represents approximately 10% of the number of cases reported before implementation of the vaccination campaign (Heinz *et al.*, 2007, Kunz, 2003).

Borodina *et al.*, (2004) evaluated the effectiveness of TBE-Moscow vaccine during the period 1999 to 2003 in the Krasnoyarsk Region, Russia, where approximately 70,000 - 105,000 people were vaccinated each year. The researchers analyzed more than 5,300 registry entries on infectious diseases and 17,400 questionnaires. More than 8,900 human sera were tested for presence of anti-TBEV antibodies. The incidence of TBE cases in 1999-2003 was found to be about 20 times lower in vaccinated than in non-vaccinated groups. Large-scale preventive measures including regular immunization of children, decreased the incidence of TBE cases per 100,000 inhabitants from 48.5 in 1999 to 6.1 in 2003. The investigators calculated also that regular vaccination in the Krasnoyarsk Region resulted in prevention of approximately 1,500 cases of TBE per year.

Also the mass-immunization programme in the Sverdlovsk Region of Russia achieved high effectiveness (*Romanenko et al., 2007*). The programme was initiated in 1996 using acell-derived vaccine produced in Tomsk. In 2000, TBE-Moscow vaccine, EnceVir, Encepur and FSME-Immune were all used in this programme, although TBE-Moscow vaccine was used in 80% of all immunizations. By 2005, 2,7 million people had been vaccinated with three doses of one of these four vaccines (*Pogodina et al., 2007*). To evaluate disease incidence, the investigators analysed the individual reporting forms on each case of TBE registered in the region during the study. Vaccination coverage increased from 35% at the beginning of the programme to 55% in 2000 and 72% in 2006, resulting in a rapid decrease in the incidence of TBE cases per 100 000 inhabitants : 42.1 in 1996, 9.7 in 2000, and 5.1 in 2006. (*Romanenko et al., 2007*). The number of cases was reduced in all age groups.

V. Immunization practice

a. Indications and contraindications

Key points (a. Indications and contraindications; b. Vaccine availability; c. Vaccination recommendations and vaccination strategies; d. Post exposure vaccination).

1. No specific contraindications to TBE vaccination except severe reaction following previous dose (documentation best for Western vaccines)
Standard precautions required as for any vaccinations.
2. TBE vaccines are available in all endemic countries
3. Recommendation for TBE vaccination vary in endemic countries: Austria only has a national vaccination program recommended for all, several countries recommend large scale vaccination in high-risk areas and/or vaccination of high risk groups; other countries have no official vaccination programme, and vaccines are given on private basis only
4. No post-exposure vaccination or other specific measure are currently recommended after a tick bite
5. For TBE, there are no recent cost effectiveness evaluations. Very few TBE-endemic countries reimburse costs for vaccination against this disease

During more than 20 years of experience, no specific contraindications for the two Western vaccines have been identified. Standard precautionary measures for vaccinations should be taken into account, however. As for all other inactivated vaccines pregnancy is considered to be a relative contraindication. And the individual risk-benefit relation has to be assessed before implementing the primary immunization series. Patients with chronic inflammatory or degenerative neurologic disease could be affected particularly severely in case of TBE and

therefore, the safety of TBE vaccination in such patients has been of special concern. Fortunately, the safety of TBE vaccination in this patient group has been confirmed.

Choosing *encephalomyelitis disseminata* (ED) as a paradigm

In a controlled clinical trial involving patients with ED, *Baumhackl et al., (2003)* investigated the longitudinal progression of their disease following vaccination against TBE. No unforeseen events and no adverse reactions attributable to TBE vaccination were observed. In particular, there were no new lesions detected by MRI. However, careful risk-benefit calculations should be performed before vaccination of these patients. There are no data from controlled trials on the safety of TBE vaccination in patients with other specific underlying diseases. As TBE vaccines are now used on a broad scale in many countries without specific precautionary measures, it is tempting to assume that TBE vaccines are safe even in patients with underlying diseases.

Although immunosuppression is not considered to be a contra-indication for TBE vaccination, the immune response may be impaired, as described for the elderly (*Weinberger et al., 2007, Hainz et al., 2005, Paulke-Korinek et al., 2009, Rendi-Wagner et al., 2004a*).

EnceVir® and TBE vaccine Moscow

According to SMPC the following contraindications should be considered:

- Acute infections – 1 month waiting period
- Chronic diseases in an acute phase
- History of severe allergic reactions, asthma, autoimmune diseases
- Gelatine hypersensitivity
- Severe complications including fever >40°C after an earlier dose of the vaccine
- Pregnancy

Details on special groups of patients or diseases are not given. The vaccines are also used for hyperimmunizations of blood donors in order to prepare a TBE-specific immunoglobulins.

b. Vaccine availability Table 8:

Availability of TBE vaccines

Encepur®	FSME-Immun®	EnceVir®	TBE vaccine Moscow®
Austria Croatia Czech Republic Denmark Estonia Finland France (adults only) Germany Hungary Latvia Lithuania Poland Romania Russian Federation Slovak Republic Slovenia Sweden Switzerland	Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Sweden and UK. Licensed using purely national routes in Canada, Croatia, Russia and Switzerland.	Available only in Russia, Ukraine, Belarus and Kazakhstan	

c. Vaccination recommendation and vaccination strategies

The recommendation for TBE vaccination are very heterogenous in European countries. An overview from 6 selected countries is given in Table 9 (adapted after *Kollaritsch et al., 2010*)

Country	TBE notifiable	TBE vaccination recommendations
Austria	✓	National vaccination program (recommendation for everyone).
Czech Republic	✓	Vaccination recommended for: <ul style="list-style-type: none"> ○ Residents in endemic areas ○ People visiting endemic areas (for recreation)
Estonia	✓	<ul style="list-style-type: none"> ▪ No national TBE vaccination policy ▪ TBE vaccination is recommended
Hungary	✓	<ul style="list-style-type: none"> ▪ Vaccination against TBE is mandatory in groups whose work puts them at a definitive risk for TBE (e.g. forestry workers and farmers, since 2000) ▪ 5–15% of the total population is immunized • Significant decrease in TBE incidence following introduction of vaccination campaign

Lithuania	✓	<ul style="list-style-type: none"> ▪ There is no official vaccination programme ▪ Vaccines are given on a private basis only ▪ Vaccination rates in Lithuania are very low
Poland	✓	<ul style="list-style-type: none"> ▪ TBE vaccination is recommended ▪ TBE vaccination rates for Poland are not officially stated; approximately 20,000 TBE vaccines are sold per year • Compulsory vaccination for forestry workers employed by National Forests (since 1994)

For details see *Donoso Mantke et al., 2008*.

Currently, only Austria has a national universal vaccination program: TBE vaccination is fully reimbursed for people with an occupational risk of TBE. For the rest of the Austrian population, the vaccine is available for the first 6 months of the year at a reduced price, physicians are charging less for its administration, and healthcare costs are partially covered by health insurance (*Kunz, 2003*). Finland, Germany, Hungary, Latvia, Slovenia, Russia and Switzerland have vaccination programmes linked to certain conditions (*Donoso Mantke et al., 2008*).

In Russia, TBE vaccination is regulated by legislative acts of the Russian Federation, including sanitary regulations 3.1.3.2352-08. According to this act, vaccination coverage of 95% in TBE endemic regions is strongly recommended. In addition, regional authorities are allowed to introduce own initiatives. The recommendations for vaccination include persons with occupational risk, tourists and visitors to endemic areas, as well as the indigenous population of the respective areas.

In specific regions with high TBE-endemicity mass-immunization programs have been conducted successfully (*Romanenko et al., 2007*).

TBE vaccination recommendation in international travel

The risk of acquiring TBE in a highly endemic area in Austria was calculated at approximately 1/10.000 per person-month (*Rendi-Wagner, 2004*).

According to *Donoso Mantke et al., (2008)* Austria, Belgium, Estonia, Finland, Germany, Greece, Latvia, Lithuania, Poland, Slovakia, Slovenia, Spain and Switzerland have recommendations for TBE-vaccination, at least for travellers to endemic areas. In 9 other EC countries there is no official recommendations concerning TBE vaccination when travelling to endemic countries.

WHO (*WHO, 2010*) recommends tick bite prevention in endemic areas during the summer months and that “Vaccine should be offered only to at-risk travellers”. Travellers are considered to be at risk when hiking or camping in rural and forested areas up to altitudes of 1.400m (*WHO, 2010*).

CDC (CDC, 2010a) recommends that particularly high- risk travellers should be vaccinated well in advance of entering endemic areas, although the vaccines are not licensed in U.S. CDC emphasizes tick bite prevention as TBE-vaccine is not available.

Other than vaccination, there are no universally accepted and commonly applied measures for prophylaxis against TBE (Banzhoff *et al.*, 2008).

For travellers, the rapid immunization schedules (see Table 7) are advantageous, since they ensure faster induction of seroconversion; antibodies in sufficient concentrations will appear as early as in week 4 after the first immunization (Rendi-Wagner, 2004; Schondorf *et al.*, 2007; Zent *et al.*, 2005).

d. Post exposure vaccination

There are no clinical studies on the possible benefit of TBE vaccination used as active post-exposure prophylaxis. Of special concern is the theoretical possibility that post-exposure prophylaxis could result in antibody-dependent enhancement of the infection and exacerbation of the disease. Such phenomena have been reported for other flavivirus infections, but not for TBEV. In the past, immune globulin treatment was recommended, but these preparations are no longer available outside Russia. Since TBE has a relatively short incubation period, even an anamnestic response may not be fast enough to protect the individual following exposure. Hence, starting vaccination after a tick bite may not in time result in appropriate neutralising antibody concentrations. For these reasons, no vaccination or other specific measure is currently recommended after a tick bite (Broker and Kollaritsch, 2008).

Persons who have received at least their basic immunization, but missed the regular boosting interval, may be boosted immediately after a tick bite, hoping that the anamnestic immune response will induce protection fast enough.

Specific immunoglobulines for post-exposure prophylaxis are no longer available outside Russia. In Western Europe, these preparations are not recommended, as there is some evidence that at least formerly available anti-TBE immunoglobulins could be of more harm than benefit (Arras *et al.*, 1996, Kaiser, 1999).

e. Economical considerations and reimbursement practices

For TBE there are no recent cost effectiveness evaluations. An estimate for Austria (Schwarz, 1993), suggests that TBE vaccine may be cost effective, at least in countries with high and widespread endemicity. TBE causes high costs for health care systems, not only for acute treatment, but even more for the care of patients with long term sequelae (Donoso Mantke *et al.*, 2008, Kaiser, 1999, Kaiser, 2008). Obviously, cost-effectiveness of TBE vaccination will

strongly be influenced by effective targeting of immunization efforts to populations at highest risk, as well as vaccine pricing. Today, very few TBE-endemic countries have started reimbursed vaccination programs against this disease (Donoso Mantke et al., 2008).

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