Tick-borne encephalitis

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We review the epidemiological and clinical characteristics of tick-borne encephalitis, and summarise biological and virological aspects that are important for understanding the life-cycle and transmission of the virus. Tick-borne encephalitis virus is a flavivirus that is transmitted by *Ixodes* spp ticks in a vast area from western Europe to the eastern coast of Japan. Tick-borne encephalitis causes acute meningoencephalitis with or without myelitis. Morbidity is age dependent, and is highest in adults of whom half develop encephalitis. A third of patients have longlasting sequelae, frequently with cognitive dysfunction and substantial impairment in quality of life. The disease arises in patchy endemic foci in Europe, with climatic and ecological conditions suitable for circulation of the virus. Climate change and leisure habits expose more people to tick-bites and have contributed to the increase in number of cases despite availability of effective vaccines. The serological diagnosis is usually straightforward. No specific treatment for the disease exists, and immunisation is the main preventive measure.

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Introduction

The first description of a tick-borne encephalitis-like disease dates back to Scandinavian church records from the 18th century. The disease was described as a clinical entity in Austria in 1931[1] and its causative agent was isolated in the eastern region of Russia in 1937. More than 10 000 cases of the disease arise every year,[2] and in terms of morbidity, this frequency is second only to Japanese encephalitis among neurotropic flaviviruses. In Europe (Russia excluded), 3000 cases are treated in hospital and reported each year,[3] with increasing numbers during the past decade.

We review here clinical and epidemiological aspects, with emphasis on the European virus subtype, and summarise the virological and biological properties of the virus that are important for the understanding of transmission and prevention.[4–7]

Virology and cellular physiology

Tick-borne encephalitis virus (TBEV) is a member of the genus flavivirus, family Flaviviridae. Flaviviruses are icosahedral enveloped 50 nm viruses with an RNA genome of about 11 kb. The TBEV genome per se acts as an infectious messenger RNA, and codes in one open reading frame for a polyprotein of 3414 aminoacids, which is co-translationally and post-translationally cleaved by viral and cellular proteases to three structural (C, prM, and E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5).[8] The C (capsid) protein, along with the viral RNA, form the spherical 30 nm capsid structure of the virus, which is covered by a lipid bilayer with two surface proteins, prM (precursor M) and E (envelope) that have double-membrane anchors. The E protein is the most important antigen and functions both as the ligand to the yet unknown receptor and as the fusion protein. The atomic structure based on crystals of TBEV E has been known for a decade.[9] E proteins are formed of three distinct domains. In the mature virus, the E proteins, which are dimerised in a head-to-tail orientation, do not form particular spikes, but lie flat on the virus surface so that the fusion peptide in the tip of the distal domain is hidden under the proximal part of the dimer partner. On cell entry by receptor recognition and endocytosis, the acidification of the endosomes triggers an irreversible conformational change. This process makes the E protein form homotrimer spikes and subsequently exposes the fusion peptide at the tip towards the endosome membrane, resulting in fusion and release of the infectious viral genome to the cytoplasm.[8] Non-structural proteins have several functions—eg, they provide the RNA-dependent RNA polymerase machinery, provide a serine protease needed to cleave the polyprotein,[10] and seem to have a role in modifying innate immune responses.[11]

Viral replication takes place in membraneous structures close to the endoplasmic reticulum, into which the virus buds and then follows the secretory pathway to exit the cell. prM acts as a chaperon for the E protein to fold correctly and protects it from ongoing premature, irreversible conformational changes during the immature virus transport through the secretory pathway. Only after cleavage of prM by the cellular protease furin, does the virus obtain a final flat, mature appearance and virions are released from the infected cell.

A special feature is that prM and E expressed together form smaller, capsid-less virus-like icosahedral particles of 30 nm. Since these particles do not have the structural protein C and RNA, they are non-infectious. These structures can form during infection or can be expressed in recombinant systems, and are highly antigenic, protect animals, and can also been used in diagnostic assays.[12,13] Another special antigen is the TBEV NS1 protein, which is partly secreted as hexameric complexes from mammalian cells, and provides full protection against

Search strategy and selection criteria

A search was done in PubMed on "encephalitis", "tick-borne" (MeSH), from 1970 and onwards. 1318 titles were screened in English or German for this Seminar. Additionally, references from the reference list of these publications were included if relevant.

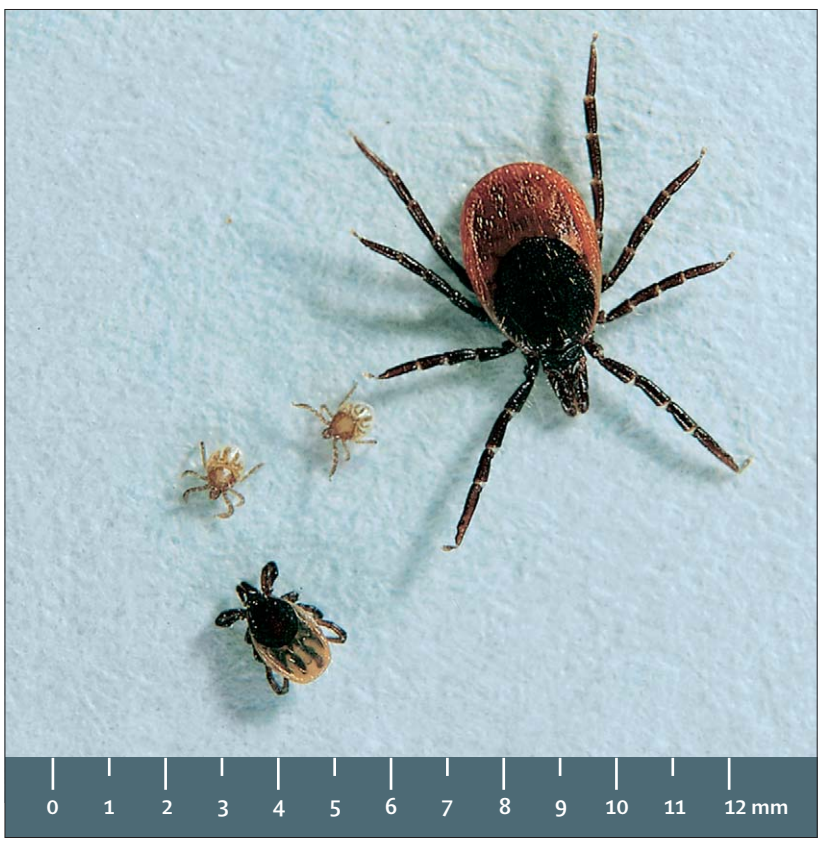

Figure 1: **Unengorged *Ixodes ricinus* ticks in different developmental stages**
From top, anticlockwise, one adult female, two larvae, and one nymph.

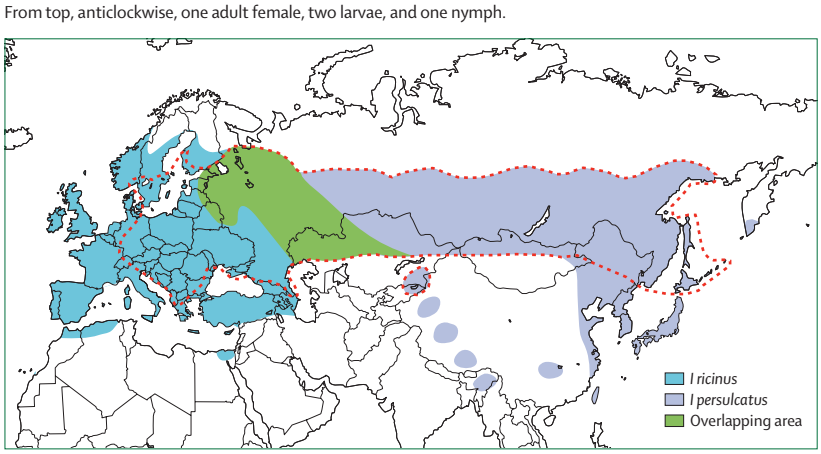

Figure 2: **The geographical distribution of *Ixodes* spp, with the western distribution for *I ricinus* and the eastern distribution for *I persulcatus***
The distribution for these two vectors overlaps in the green area. The dotted line defines the border for the tick-borne encephalitis endemic area. Note that the disease is very focally distributed within its endemic zone. Ixodes distribution in China is uncertain.

infection in immunised animals and raises antibody responses in infected people.[14]

Because the RNA genome is infectious, the viral genome can be mutated and manipulated by its transcription from DNA plasmids. This process has led to direct approaches for making tailored attenuated live viruses—eg, with flavivirus chimeras, attenuated TBEV mutants, modified virus particles able to do only one round of cell infection, and DNA or RNA vaccines.[15] However, vaccines at present are traditionally purified formalin inactivated virions, consisting of C, M, and E proteins that can undergo slight conformational changes during the inactivation process and therefore deviate somewhat from their fully native state.

Evolution and epidemiology

Other medically important flaviviruses include the mosquito-borne yellow fever, dengue (DENV 1,2,3,4), Japanese encephalitis, and West Nile viruses. The substantial homology between these viruses has practical implications in diagnostics because of cross-reactions. Phylogenetic analyses have shown that tick-borne flaviviruses are distinct from mosquito-borne viruses and seem to evolve more slowly[16] because of the tick's long lifespan of usually about 2 years. Unlike mosquito-borne viruses, no evidence of genetic recombination has been obtained for tick-borne flaviviruses so far.[17] Tick-borne viruses can be further divided into groups related to mammalian or sea-bird hosts. TBEV belongs to the mammalian group along with rare human pathogens, such as the Powassan and Omsk haemorrhagic fever viruses, which seem to represent more ancestral lineages.[17,18]

TBEV consists of three subtypes: (1) the European (TBEV-Eu); (2) Siberian (TBEV-Sib); and (3) Far Eastern (TBEV-FE). The vector of TBEV-Eu is *Ixodes ricinus* (figure 1), and *I persulcatus* for the other two subtypes.[6,7,18–22] Within a subtype, variation in aminoacids is up to 2·2% and 5·6% between subtypes.[19] *I ricinus* is seen in most of Europe, and the distribution extends to Turkey, northern Iran, and Caucasus in the southeast (figure 2).[23] *I persulcatus* is found in the belt extending from eastern Europe to China and Japan. Both tick species cocirculate in a restricted area in northeastern Europe, Russian Karelia, St Petersburg, and eastern Estonia and Latvia.[22,24] Consequently, all three TBEV subtypes have been recorded in the regions. Additionally, an ectopic focus of TBE-Sib carried by *I persulcatus* has been discovered in western Finland.[25]

The diversity of TBEV carried by *I persulcatus* is much higher than for the other two species and has probably been evolving for thousand(s) of years, whereas the TBEV-Eu strains in *I ricinus* are very similar, do not show clear geographical clustering (ie, strains within a country do not form genetic groups), and have spread in past few centuries[16,17,24–26] possibly by migratory birds.[27] TBEV-Eu is more closely related to the louping ill virus, also carried by *I ricinus* (sheep tick), but which rarely infects man.[17]

TBEV is transmitted from the saliva of an infected tick within minutes of the tick-bite. Although the virus in tick saliva increases ten-fold to 100-fold during feeding,[28] early removal of ticks does not prevent disease. Tick-borne encephalitis can occasionally be transmitted after an intake of unpasteurised milk

products from viraemic livestock. Large outbreaks from a common source are associated with dairy products, but are of less importance than they were. However, minor outbreaks are still reported.[29] Single cases of tick-borne encephalitis after slaughter of probably viraemic goats,[30] blood transfusions,[31] breastfeeding,[32] and laboratory investigations[33] have also been described.

In Europe, tick activity starts in spring when the temperature approaches 6°C and usually persists until November when the temperature falls.[6,34] In central Europe, a two-peak distribution of cases in early and late summer can be seen,[35] lagging 2–4 weeks behind the bimodal peak of tick activity. Studies from central Europe[36,37] showed a monophasic distribution, resembling the situation in northern Europe with one peak during July and August.[38] Human habits strongly influence the incidence and seasonal distribution.[39] Frequently, the disease is contracted during leisure time[6,40] in the hot summer months despite lower than average tick activity. Socioeconomic circumstances and reduced arable acreage can increase exposure. In the Baltics and elsewhere in the former USSR, retired and unemployed people are at increased risk because of activities such as berry and mushroom picking.[38] Owners of summer houses in endemic areas are also a risk category.[38,41] Tick-borne encephalitis acquired during travel could be an underdiagnosed problem, but whether the risk for indigenous populations can be translated into risk for travellers is unclear. In prospective studies, a predominance of infection in men is usually seen in all age groups.[36–38,42] Previously, a high incidence was seen in some professional groups, such as forest workers, but immunisation has changed this situation.

The disease arises in a very large geographical area (figure 2). During the past two decades, both new endemic foci[6] and an increase in cases have been reported in many European countries[3] (table 1), with the major exception being Austria, which has a high vaccine coverage (>86%).[43] A practical consequence of this emerging situation is that the disease also has to be considered outside the traditional endemic areas. Some reports of new endemic areas are attributed to a previous underdiagnosis of cases.[44] However, the true nature of this rise is supported by an increase in areas with high awareness of the disease and with well established diagnostic routines.

The ecological specifications to maintain a natural environment for TBEV are complex[6,34] and fragile.[45] Suitable temperature (6–25°C) and humidity (>85%), specific biotypes such as meadows and forests with rich undergrowth, and favourable density of hosts determine abundance of ticks. TBEV is, unlike *Borrelia burgdorferi*, not found throughout the range of *I ricinus* and is therefore dependent on additional environmental

	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006*
Albania	8	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Austria	89	128	84	102	178	109	128	99	62	41	60	54	60	82	54	100	84
Belarus	..	..	2	20	50	66	97	67	78	26	23	61	18	25	..	..	..
Croatia	23	60	27	76	87	59	57	25	24	26	18	27	30	..	..	..	20
Czech Republic	193	356	338	629	613	744	571	415	422	490	719	411	647	606	500	642	1·113†
Denmark	..	..	..	..	..	..	..	..	1	4	3	1	1	4	8	4	..
Estonia	37	68	163	166	177	175	177	404	387	185	272	215	90	237	182	164	171
Finland	9		14	25	16	23	10	19	17	12	41	33	38	16	31	17	18
France	2	1	2	5	4	6	1	1	2	5	0	0	2	6	7	0	..
Germany	..	44	142	118	306	226	114	211	148	115	133	253	226	278	274	431	547†
Hungary	222	288	206	329	258	234	224	99	84	51	45	76	80	114	59	90	115
Italy	..	..	2	2	8	6	8	8	11	5	15	19	6	14	23	22	14†
Latvia	122	227	287	791	1·366	1·341	716	874	1·029	350	544	303	153	365	251	142	171
Lithuania	9	14	17	198	284	426	309	645	548	171	419	298	168	763	425	242	462
Norway	..	..	..	..	..	..	..	..	1	1	2	1	2	1	3	0	5
Poland	8	4	8	249	181	267	257	201	209	101	170	205	126	339	262	174	316†
Russia	5·486	5·225	6·301	7·893	5·593	5·982	9·548	6·539	6·987	9·955	5·931	6·339	5·150	4·770	4·235	4·551	3·510†
Slovakia	14	24	16	51	60	89	101	76	54	57	92	76	62	74	70	28	..
Slovenia	235	245	210	194	492	260	406	274	136	150	190	260	262	275	204	297	372†
Sweden	54	75	83	51	116	68	44	76	64	53	133	128	105	105	160	130	163
Switzerland	26	37	66	44	97	60	62	123	68	112	91	107	53	116	138	206	259
Ukraine	..	..	..	..	..	..	..	..	..	..	..	..	12	..	..	..	..

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***Table 1*: Number of reported cases of tick-borne encephalitis from European countries and Russia**

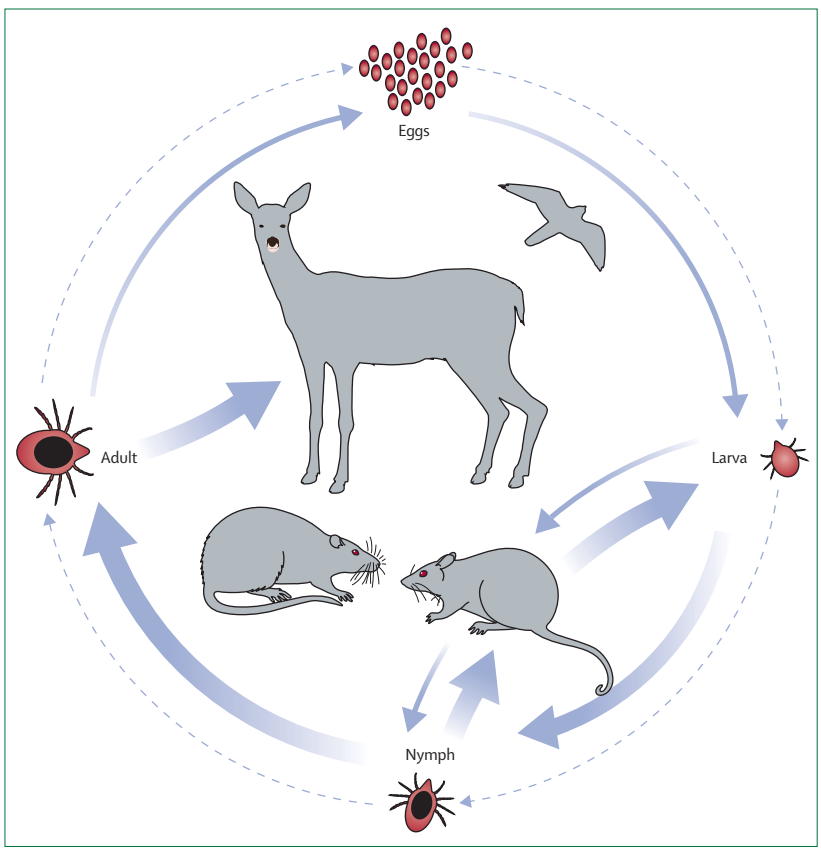

Figure 3: **Transmission cycle for tick-borne encephalitis virus**
Dashed line=different developmental stages of tick in cycle (clockwise from top: eggs, larvae, nymph, and adult). At each stage tick needs blood meal to develop into next stage whereby it feeds on suitable host. Additionally, adult females need blood meal to produce eggs. Solid lines=presence of tick-borne encephalitis virus (TBEV) in tick (which after being infected carry virus throughout its lifespan, including after occasional transovarial transmission); main transmission event enabling maintenance of a European subtype TBEV focus arises from nymphs to larvae cofeeding on the same rodent host. Thickness of arrows shows the prevalence of the tick.

factors that restrict its presence to patchy foci from a few square metres to several square kilometres in size.[6,46] Because of its long lifespan, the tick is the main reservoir for the virus.[47] Viral transmission to a tick takes place when ticks at different developmental stages (particularly nymphs and larvae) co-feed on the same animal—typically yellow-necked mice or other rodents. The virus is transported non-viraemically, even in an immune host, to the next tick generation (figure 3).[48–50] Transmission could also take place through viraemic animals,[51] or occasionally (less than 0·5%) transovarially from infected females to eggs.[52] For effective TBEV-Eu transmission, synchronous activity of larvae and nymphs supported by specific climatic conditions—for instance, rapid warming in springtime—are needed.[53,54] Reduced biodiversity could also increase viral transmission in favourable hosts.[55] In the developmental stages of *I ricinus*, nymphs are most abundant and less host specific, and therefore most important in human transmission[6] whereas for *I persulcatus* adult ticks are more important in human transmission.

Environmental factors important for sustaining natural foci for tick-borne encephalitis can be modelled with satellite data.[45,54] These models suggest that climate change is partly responsible for increased incidence in Europe. This hypothesis is supported by longitudinal studies from the Czech Republic,[56] showing TBEV circulation at increasing altitudes. In Sweden, a northward expansion of *I ricinus*[57] is seen, and mild winters and early spring are associated with an increase in incidence of the disease.[58] WHO has predicted global warming to increase vector-borne diseases.[59] However, the virus could also disappear if high temperatures and low humidity prevail, and studies from the Baltics have failed to fully correlate increased incidence with climate change.[60]

Existing methods for risk assessment have limitations. Increasing coverage for vaccination and difficulties in defining the exposed population could hamper methods based on case notification and seroprevalence.[43,61] The risk of an unvaccinated person contracting the disease in Austria[43] is probably higher at present than it was 30 years ago, despite a 90% fall in incidence. In Europe, TBEV prevalence varies between 0·1% and 5% in ticks identified by reverse transcriptase (RT)-PCR, with an increasing prevalence during the life-cycle of the tick,[40] and is up to 10% in engorged ticks removed from individuals. The highest tick prevalence is usually seen in highly endemic areas, but surveillance methods need careful standardisation with long-term follow-up to be informative.[40] Longitudinal data from the Baltic states suggest that environmental variables explain 55% of the variance in the incidence of tick-borne encephalitis.[62] Therefore, risk assessment should combine human habits and socioeconomic variables with functional variables in natural foci. Seropositivity in vertebrate hosts is caused by frequent exposure to ticks, which is a sensitive indicator of TBEV in nature, but might not be directly translated into a risk for people. Moreover, remote sensing and mathematical modelling[63] have provided us with improved methods to map risk areas for TBEV, and have already predicted new foci in Europe.

Clinical presentation and pathogenesis

Tick-borne encephalitis follows an incubation period of a median of 8 days (range 4–28) after tick bite,[36] which is unnoticed in about a third of patients.[36,38,64] Typically, the disease is biphasic in 72–87% of patients.[36,38,64] The median duration of the first stage of illness is 5 days (range 2–10) with a 7 day (range 1–21) symptom-free interval to the second phase. In the first viraemic stage, the dominant symptoms are fever (99%), fatigue (63%), general malaise (62%), and headache and body pain (54%).[38] Leucopenia and thrombocytopenia and slightly raised serum transaminases can be seen in this first stage, although leucocytosis is frequent in the second stage.[36,65] Seroconversion without prominent morbidity is common. In a prospective field study in adults in a highly endemic

	Duniewicz et al[68]	Falisevac et al[69]	Radsel-Medvescek et al[70]	Krech et al[71]	Jezyna et al[72]	Kaiser[36]	Grygorczuk et al[73]	Mickiene et al[38]	Wahlberg et al[74]
Number of patients	589	1218	315	234	215	656	152	133	301
Headache	67%	..	100%	74%	100%		84%	95·5%	81·7%
Altered consciousness	..	..	13·7%	29%	35·5%	31%	24%	18·8%	12%
Sensory impairment	..	..	..	9%	..	2·9%	2%	..	..
Seizures	0·3%	..	..	2%	3·3%	1·7%	..	..	..
Ataxia	30%	..	..	..	..	18%	24%	26·3%	0·3%
Hemiparesis	..	0·3%	..	..	..		1·9%	2·6%	0·3%
Tremor	75%	..	78%	..	31·6%	4·3%	7%	21·8%	..
Dysphasia	..	..	..	..	..	2·5%	0·7%	3·8%	..
Spinal nerve paralysis	12·8%	2·7%	6·3%	10%	8·8%	15%	7·2%	3·8%	4·3%
Cranial nerve paralysis	..	..	3·5%	..	..	11%	3·3%	5·3%	..

..= data not given.

Table 2: **Summary of neurological symptoms in the acute stage of tick-borne encephalitis in studies including a minimum of 100 patients**

area in Sweden, with a yearly seroconversion rate of 1·2–2·4%, only 25% developed severe disease.[66,67]

In the second stage, the clinical spectrum ranges from mild meningitis to severe encephalitis with or without myelitis and spinal paralysis. Neurological symptoms at this stage do not, in principle, differ from other forms of acute viral meningoencephalitis (table 2).[36,38,68–74] Seizures are infrequent but altered consciousness is seen in a third of patients. In Kaiser's study,[36] 12% of those with decreased consciousness had a Glasgow coma scale less than 7.

Cerebrospinal fluid (CSF) analyses reveal moderate pleocytosis,[36,38,64] with two-thirds of patients having 100 leucocytes per µL or less. An initial predominance of polymorphonuclear cells is later changed to an almost 100% mononuclear cell dominance. Two-thirds have a moderate increased CSF albumin, peaking at a median day 9. Objective meningeal signs could be absent in about 10%, despite CSF pleocytosis.[73] Therefore, patients presenting only with fever as the prominent symptom without encephalitic signs could be suspected as having some other infectious syndrome. Abnormalities on MRI are seen in up to 18% with lesions usually confined to the thalamus, cerebellum, brainstem, and nucleus caudatus.[75,76] Electroencephalogram (EEG) is abnormal in 77%.[36] Both MRI and EEG abnormalities are unspecific and not diagnostic.

As a result of a preference for the anterior horn of the cervical spinal cord, a flaccid poliomyelitis-like paralysis arises that, unlike poliomyelitis, usually affects the arms, shoulders, and levator muscles of the head. In about 5–10% of cases, monoparesis, paraparesis, and tetraparesis can develop, as well as paralysis of respiratory muscles, requiring ventilatory support.[36] Cranial nerve involvement is mainly associated with ocular, facial, and pharyngeal motor function, but vestibular and hearing defects are also encountered. In severe cases, brainstem involvement can lead to substantial respiratory and circulatory failure. An isolated bulbar syndrome without myelitis has also been described, usually with a fatal outcome.[38,77] Apart from myelitis, tick-borne encephalitis can develop into a myeloradiculitic form, typically presenting a few days after defervescence, and could be accompanied by severe pain in the back and limbs, weak muscle reflexes, and sensory disturbances. Paralyses could develop that, compared with myelitis, have a more favourable prognosis.[78]

In two prospective studies, with the same clinical definitions, severe forms of encephalitis were seen in 44–55% of adults.[38,64] From these studies we can clearly deduce that spinal nerve paralysis is partly dissociated from CNS parenchymal involvement and could be seen in patients without encephalitis. In two large prospective studies[36,42] of children and adults, major increase in severity of illness was seen with increasing age. A substantial increase in morbidity in elderly people (figure 4) makes them a special target group for immunisation.

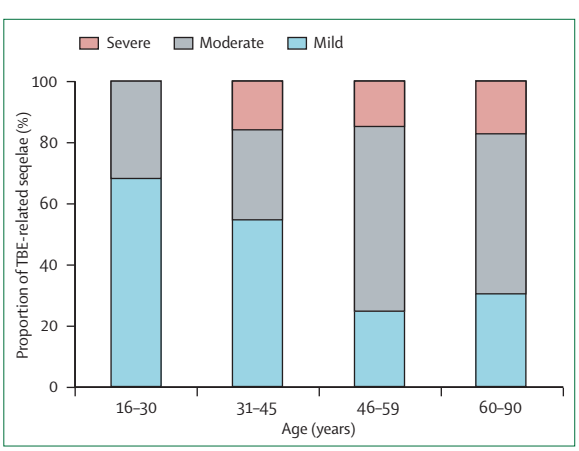

Figure 4: **Age-related proportion of mild, moderate, and severe form of tick-borne encephalitis in a prospective study from Lithuania[38]**
Clinical presentation of meningoencephalitis at onset of disease is classified as follows: mild=mainly meningeal symptoms; moderate=monofocal symptoms of the CNS and moderate diffuse brain dysfunction, or both; severe=multifocal CNS symptoms and severe diffuse brain dysfunction, or both. TBE=tick-borne encephalitis.

Retrospective follow-up studies[41,68,69,72,74,79–81] report a case-fatality rate of 0–1·4% and acute and residual spinal paralysis in 3–13% and 0·3–6·8%, respectively, which is well in accordance with prospective studies. These findings suggest that various residual cognitive symptoms are common, but study design does not allow any firm conclusions.

Four prospective follow-up studies[36,38,42,64] have assessed the long-term morbidity that is associated with TBEV-Eu. A consistent finding is the existence of a postencephalitic syndrome. In the three studies[38,42,64] with few patients lost during follow-up, 26–46% reported some form of remaining symptoms after 6–12 months (table 3). Neurological dysfunction was associated with moderate to severe impairment in quality of life in 30%.[38] The risk of incomplete recovery was also very high for patients who had moderate to severe tick-borne encephalitis in the acute stage (odds ratio 4·1, 95% CI 1·8–8·9). Whether sequelae present at follow-up after 1 year would improve with time is unknown. However, in a retrospective study[41] of 114 consecutive patients with a mean follow-up time of 4 years, 28% had moderate to severe sequelae with the same classification as in the Lithuanian study.[38] These findings show that sequelae present after 1 year indicates a poor prognosis.

	Günther et al[64]	Tomazic et al[42]	Mickiene et al[38]
Study details			
Number of patients	85	492	133
Year of patient enrolment	1991–93	1994	1998–99
Number of patients lost at follow-up (%)	2 (2·3%)	6 (1·2%)	15 (11·4%)
Follow-up	12 months	6 months	12 months
Control group	Other viral meningoencephalitis	No	Healthy controls (neuropsychiatric questionnaire)
Reported sequelae at end of follow-up			
Total with incomplete recovery	39·8 %	26·1%	46·2%
Headache	10·8%	22·6%	20·5%
Concentration difficulties	8·4%	15·2%	15·4%
Memory impairment	10·8%	..	19·7%
Emotional instability	..	..	18·8%
Fatigue	..	21·7%	..
Light and sound irritability	1·2%	..	..
Mental disturbance	..	1·4%	..
Consciousness disturbances	..	0·2%	..
Sweating	..	5·5%	..
Sensory disturbance with pains and dysaesthesia	2·4%	11·2%	..
Ataxia and tremor	9·6%	10·2%	14·5%
Dysphasia	6·0%	..	..
Hearing loss	2·4%	..	..
Spinal nerve paralysis	6·0%	2·2%	6·0%
Case-fatality rate	0%	0·2%	0·8%

.. = data not given.

Table 3: **Neurological sequelae at follow-up in prospective studies on patients with tick-borne encephalitis**

Although the severity of the acute stage of the disease is closely related to age, our knowledge of long-term morbidity in children, especially very young children, is incomplete. Severe disease associated with TBEV-Eu in children younger than 3 years is rare.[82–84] Table 4 shows case series in children up to 15 years of age, in whom severity of illness by age was reported. Of the 1144 children in these series,[36,37,42,84–90] one aged 5 years had major neurological sequelae whereas ten (0·9%) children aged 7 years or older had neurological sequelae. Permament neurological sequelae has been reported elsewhere in two additional children younger than 7 years infected with TBEV-Eu.[32,91] An unfavourable course has also been described in five children aged 1–14 years after the use of specific hyperimmune globulin as postexposure prophylaxis after tick-bite.[31,92,93] In these five children, antibody-mediated enhancement might have contributed to the aggravated course. One should remember the difficulty in assessing cognitive disturbances in children. A small follow-up study,[94] in which young children who had recovered from the disease did badly in neuropsychological testing, shows that morbidity in childhood is underestimated and calls for further follow-up studies.

Age, severity of illness in the acute stage, and low neutralising antibody titres at onset are associated with severe forms of the disease,[95] along with low early CSF IgM response.[96] The degree of virus neutralising capacity can determine the degree of viraemia that is experimentally associated with development of disease.[7,97] Both monophasic disease and short biphasic course have also been associated with severe disease.[36,38] In the study by Kaiser,[36] but not by others,[38,64] a pronounced CSF cellular response was associated with an unfavourable outcome.

The route and mechanism of entry of TBEV into the brain is believed to be haematogenic, but data are conflicting.[98] Only one study[99] of immunohistochemical visualisation of viral antigen in patients who died has been reported. Viral antigen was identified in 20 of 28 brains, predominantly in large neurons with widespread localisation in the same areas as pathological changes were seen with neuroimaging in patients.[75,76] A poor topographical relation was noted between inflammatory changes, mainly T cell and macrophages, and distribution of antigen, suggesting an immunomediated neuronal cell death rather than direct viral lysis. Attempts to characterise the inflammatory response[100] have shown a prominent inflammatory T-cell response in the CSF, but failed to elucidate the mechanisms behind brain damage and dysfunction in tick-borne encephalitis.

Studies have associated TBEV-FE with more severe disease than with the other subtypes and a case-fatality rate of up to 20–40%.[7,101,102] However, on the basis of the difference in seroprevalence rate in Europe (1–20%) and in Russia (30–100%),[6,101–104] this difference in morbidity could, at least partly, be due to a selective registration of mainly severe cases. In studies from western Siberia,[105] where the TBEV-Sib is prominent, the reported case-fatality

rate was 2–3%. The poliomyelitic form, diagnosed in up to 36% of cases in the 1940s, was only occasionally seen. Higher severity of illness in preschool children associated with TBEV-FE still shows a higher morbidity with this subtype than with the other subtypes, but the difference could be less pronounced than previously described. Reports of chronic and progressive forms of the disease, especially with TBEV-Sib, are described in Russian publications.[7] Both mutations in the TBEV *NS1* gene[106] and a defective T-cell response[107] have been associated with chronic progressive disease. However, progressive forms are very unusual with TBEV-Eu. Only two cases have been reported,[38] both were RT-PCR negative in CSF. Eight patients with fatal haemorrhagic syndrome were recorded in the Novosibirsk region of Russia, where the virus isolated from brain tissue clustered with the TBE-FE subtype.[108] Animal studies, including primates, support a high neurovirulence of TBEV-FE and persistent infections with TBEV-Sib.[7] Molecular determinants of pathogenicity, and their variation within and between subtypes, need to be established.

Laboratory diagnosis

The diagnostics of TBEV are straightforward: as a rule, TBEV-immunoglobulin M (IgM) and usually TBEV-IgG antibodies are present in the first serum samples taken when CNS symptoms manifest in the second phase of the disease. In the first phase of illness, the virus can be isolated or detected by RT-PCR from blood, but only rarely is TBEV detected at the beginning of the second phase in CSF[109] and occasionally in cases of progressive disease.[106] Intrathecal IgM and IgG antibody response can be detectable in CSF, but several days later than in serum, and in all cases by day 10.[96,110] Enzyme immunoassays are usually used for specific serodiagnosis; these assays could be based on either purified virions or recombinant virus-like particles obtained by expression of prM and E proteins.[13] Also haemagglutination inhibition is widely used, but measures all antibody classes and needs a rise in antibody titre for definitive diagnosis. Because of high cross-reactivity of the antigenic structure in the flavivirus, possible diagnostic difficulties could arise in areas where other flaviviruses cocirculate (eg, West Nile virus in the southern parts of the tick-borne encephalitis endemic area), or when the person has travelled recently in, for example Japanese encephalitis or dengue virus endemic areas, or has been vaccinated against TBEV, Japanese encephalitis, or yellow fever viruses. In such cases, detection of TBEV-specific antibodies in CSF and neutralisation studies (requiring biosafety level 3 laboratories) with convalescent serum samples are needed to establish the diagnosis of tick-borne encephalitis with certainty. IgM responses are also generally type-specific. In cases of suspicion of a vaccine breakthrough, a second sample showing a delayed rise in antibody titre or a positive IgM, or presence of a specific CSF response is needed for diagnosis.

	Cases	Age of children (years)	Severe acute disease	Sequelae
Harasek[84]	38	7–14	2 children (11 and 12 years) with myelitis	1 child (age not given) on antiepileptic drugs for 1 year
		<7	0	0
Falk el al[85]	80	7–15	5	0
		<7	0	0
Helwig et al[86]	13	7–15	3	0
		<7	1 (2 years old with seizures)	0
Messner[87]	93	7–15	2	0+1 fatal (11 years old)
		<7	1 (2 years old with seizures)	0
Rakar[88]	146	7–15	15	6 severe sequelae (2 spinal paralysis, 3 seizures, 1 behavioural changes)
		<7	0	0
Cizman et al[89]	133	7–14	6 (treated in ICU)	3
		<7	1 (6 years old) treated in ICU	0
Kaiser[36]*	77†	<15	9 had impaired consciousness, 2 had spinal paralysis, and 3 cranial nerve paralysis	Not clear if any sequelae (not reported)
Tomazic et al[42]*	77†	≤15	1 child (age not given) with myelitis	Not clear if any sequelae (not reported)
Lesnicar et al[37]*	371	≤15	11 had transitory spinal paralysis and 14 transitory cranial nerve paralysis. None required ICU	0
Fritsch et al[90]*	116	≤15	9 (2 seizures, 2 hearing impairment, 1 facial palsy, 1 hemiparalysis, 2 major concentration difficulties, 1 dyslalia)	2 (1 with seizures [7 years old] and 1 with permanent hemiparesis [5 years old])

ICU=intensive care unit. *Studies in which the exact age of children with severe disease is not specified. †Prospective studies.

Table 4: **Prospective and retrospective studies of tick-borne encephalitis in children, by severity of illness and age**

Treatment and prophylaxis

No specific treatment for tick-borne encephalitis exists. In a large German study,[36] 12% of patients needed intensive care and 5% assisted ventilation. The use of corticosteroids is not supported by any controlled study or uncontrolled studies.[38] No established treatment exists for chronic progressive forms. However, one progressive case from Lithuania[38] responded to corticosteroid treatment and plasma exchange. This finding suggests the existence of two categories of progressive disease: (1) one without TBEV presence that responds to immunomodulatory intervention; and (2) another with TBEV presence in which antiviral treatment would be rational, if available.

Tick-borne encephalitis can be prevented by active immunisation.[111] Apart from the Russian vaccines based on TBEV-FE, two vaccines based on almost identical TBEV-Eu strains (strain Neudoerfl, FSME-IMMUN by Baxter Vaccines, Vienna, Austria; strain K23, Encepur by Novartis, Basel, Switzerland) are licensed in Europe. In animals, cross-protection between major subtypes of TBEV are induced.[112,113] Both vaccines have since their introduction (1976 for FSME-IMMUN and 1991 for Encepur) undergone modifications. Viral antigens are propagated in chick

embryo cells, filtered and inactivated by formaldehyde, and further purified by ultracentrifugation. The antigen is adsorbed to aluminium hydroxide and stabilised with human albumin (FSME-IMMUN) or sucrose (Encepur), and is free from thiomersal.

No controlled trials have been done to show their protective efficacy. After the start of mass vaccination in Austria, resulting in a major decrease in the expected number of cases, a rate of protection of over 95%[43] could be estimated for FSME-IMMUN. Conventional immunisation schedules are similar for both vaccines, with two intramuscular doses given 1–3 months apart before the period of transmission and a third dose given before the next tick season. This schedule induces for both vaccines[111,114–122] antibody concentrations that are believed to be protective in over 90% of children and adults. However, occasional vaccine breakthroughs have been reported.[123,124] The protective amount of antibodies is not clearly defined and standardised, making comparisons between vaccines difficult. A good correlation between different methods for determing antibody amounts exists.[125] Since vaccine breakthroughs have been attributed to the absence of antibodies to neutralising epitopes, despite the presence of specific antibodies detected with immunoassays,[126] neutralising antibody activity is the best surrogate marker for protection. An ELISA antibody concentration of 126 Vienna units or more[126] also suggests immunity if exposure to other flaviviruses can be ruled out.

On the basis of the homology of the antigen and demonstrated cross-boostering,[127] the two vaccines seem interchangeable after the induction of a primary immune response. The adult antigen content in FSME-IMMUN and Encepur is 2·4 µg and 1·5 µg, respectively, and half this dose is used in paediatric formulations. The immune response after vaccination is age dependent, with children having an enhanced immune response compared with adults,[128] whereas older age groups, especially over 60 years, frequently have a poor antibody response.[129] Because of the gradual decline of antibodies after the third dose, a booster is needed for both vaccines after 3 years. After the fourth dose, a more stable antibody concentration is maintained in most individuals,[127,130] allowing a long booster interval of 5 years. Whether booster intervals after the fifth dose can be further extended has not been fully analysed. A special case is that of elderly people over 60 years of age for whom the 3-year-booster interval is advisable. However, since low responders and vaccine breakthroughs are more frequent in the older age group,[43,131,132] a need for an additional dose for elderly people in the primary series needs to be addressed. Accelerated schedules for use during the endemic season have been introduced for FSME-IMMUN, with a shortened interval down to 2 weeks between the first two doses,[133] and for Encepur three doses given on days 0, 7, and 21.[134] Accelerated two-dose schedules result in a reduced mean of antibody titres and seroconversion rate.[135] It is worth emphasising that the experience with tick-borne encephalitis vaccines is based mainly on conventional schedules, which should be chosen if no important time constraints exist.

Few studies have examined the health economic aspect of immunisation for tick-borne encephalitis and the threshold in incidence in which costs and benefits balance is unknown. The immunisation campaign in Austria[136] had an estimated yearly benefit in the 1990s that was equivalent to US$80 million on the basis of morbidity prevented, but without taking costs for vaccination into account. In Sweden the cost-effectiveness ratio for immunisation was estimated to be 1·68, if hypothetically 75% of cases were averted by vaccination of 42% of the Stockholm population.[41]

Several studies and the widespread postmarketing experience with tick-borne encaphalitis vaccines available in Europe have shown good tolerance and safety. A few reports of neurological adverse events, mostly neuritis, have been published.[137,138] Vaccines are more reactogenic in children, necessitating the reduced antigen content in paediatric formulations.[128,139] Mild or moderate febrile reactions take place in 15–20% of patients, especially after the first dose and in young children.[140,141] Serious systemic reactions are very rare.

Passive immunisation with hyperimmune IgG against TBEV has been frequently used in some countries as postexposure prophylaxis. Because of the absence of well documented effectiveness and fear of inducing an exacerbation of the clinical course[91,92] by immunomediated enhancement,[93] this immunisation can no longer be recommended.

Conflict of interest statement

We declare that we have no conflict of interest.

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