Tick-borne encephalitis virus – a review of an emerging zoonosis

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During the last 30 years, there has been a continued increase in human cases of tick-borne encephalitis (TBE) in Europe, a disease caused by tick-borne encephalitis virus (TBEV). TBEV is endemic in an area ranging from northern China and Japan, through far-eastern Russia to Europe, and is maintained in cycles involving Ixodid ticks (Ixodes ricinus and Ixodes persulcatus) and wild vertebrate hosts. The virus causes a potentially fatal neurological infection, with thousands of cases reported annually throughout Europe. TBE has a significant mortality rate depending upon the strain of virus or may cause long-term neurological/neuropsychiatric sequelae in people affected. In this review, we comprehensively reviewed TBEV, its epidemiology and pathogenesis, the clinical manifestations of TBE, along with vaccination and prevention. We also discuss the factors which may have influenced an apparent increase in the number of reported human cases each year, despite the availability of effective vaccines.

Introduction

Tick-borne encephalitis virus (TBEV) is the causative agent of tick-borne encephalitis (TBE), a potentially fatal neurological infection affecting humans in Europe and Asia (Gritsun et al., 2003a). From 1974 to 2003, a 400% increase in TBE morbidity had been observed in Europe (Süss, 2008), and TBEV can now be found in regions that were previously unaffected (Charrel et al., 2004). TBE is now a notifiable disease in 16 European countries (Süss, 2008) and cases have been confirmed in areas where it has not been previously reported, for example Norway (Csango et al., 2004). Between 1990 and 2007 there were an average of 8755 reported cases of TBE per year in Europe and Russia, in comparison with an average of 2755 per year between 1976 and 1989 (Süss, 2008). This increase may have been caused by an expanding tick population, promoted by factors including climate change, social and political changes and changes in land use (Süss, 2008). However, a number of European countries have observed a reduction in the number of cases in recent years, including Austria, which ran a successful vaccination campaign introduced in 1981 (Fig. 1) (Süss, 2008). The overall figures for Russia also suggest a similar trend, although the numbers of cases are still in the thousands (Fig. 1) and in one region of Russia alone (Ekaterinburg, Ural region), there are 300–500 reported cases every year (Toporkova et al., 2008). Within Russia, western Siberia has the highest incidence of TBE cases in the world (10,298 cases in 1996), although the apparent yearly decrease to 3098 cases in 2007 cannot be attributed to vaccination alone (Süss, 2008). In terms of morbidity of neurotropic flavivirus infections, these figures are second only to those for Japanese encephalitis (Lindquist & Vapalahti, 2008). Although Russia has by far the greatest number of confirmed TBE cases (Süss, 2008), the Czech Republic has one of the highest incidence rates in Europe, with 400–1000 clinical cases reported annually (Růžek et al., 2008). Virulence of TBEV for humans has been shown to decrease with its westward spread from Japan/far-eastern Russia, through central Europe to western Europe (Gritsun et al., 2003a), with the far-eastern subtype having a case fatality rate as high as 40% (Mandl, 2005).

Virology and epidemiology of TBEV

TBEV is a member of the genus Flavivirus, within the family Flaviviridae (Mandl et al., 1997). It is a member of the mammalian tick-borne flavivirus group, which, along with other genetically and antigenically related viruses, includes Omsk hemorrhagic fever virus (OHFV), Langat virus (LGTV), Alkhurma hemorrhagic fever virus (AHFV), Kaysanur Forest disease virus (KFDV), Powassan virus (POWV), Royal Farm virus (RFV), Karshi virus (KSIV), Gadgets Gully virus (GGYV) and Louping ill virus (LIV)
Flaviviruses, including TBEV, are small, lipid-enveloped viruses with a spherical structure and a diameter of 40–60 nm (Lindenbach & Rice, 2001). The flavivirus genome comprises a single-stranded, positive-sense RNA of approximately 11 kb in length, containing a 5’ cap and lacking a polyadenylate tail (Wengler et al., 1978). The 5’ cap is important for mRNA stability and translation (Furuichi & Shatkin, 2000). The single open reading frame (ORF) encodes three structural proteins – capsid protein C, membrane protein M (formed by cleavage from its precursor prM) and the large envelope glycoprotein (E) – along with seven non-structural proteins – NS1 (glycoprotein), NS2A, NS2B (protease component), NS3 (protease, helicase and NTPase activity), NS4A, NS4B and NS5 (RNA-dependent polymerase). The 5′ and 3′ ends of the genome are non-coding regions (reviewed by Chambers et al., 1990a). The viral genome RNA is self-infectious and would produce virus progeny if introduced into susceptible cells (Mandl et al., 1997). Many studies have focused on the TBEV E protein, which is known to exist as flat dimers extending in a direction parallel to the viral membrane, with residues important for antibody binding exposed on the outer surface of the protein (Rey et al., 1995). The second transmembrane region of the E protein is known to be important for virion formation (Orlinger et al., 2006). The E protein also has the ability to form enveloped recombinant subviral particles (RSPs) with prM (Allison et al., 1995a), which assemble in the endoplasmic reticulum (ER) and are transported through the secretory pathway in the same way as whole virus particles (Lorenz et al., 2003). Cryoelectron microscopy has shown that mature TBEV RSPs have an icosahedral molecular organization (Ferlenghi et al., 2001). The flavivirus NS3 protein is multifunctional, including the C terminus region which possesses both nucleoside triphosphatase (NTPase) and helicase activities (Wenger & Wenger, 1991). The N-terminal region of the NS3 protein possesses protease activity, which is necessary for the post-translational cleavage of the viral polyprotein precursor (Chambers et al., 1990b). Studies with West Nile virus (WNV) have suggested that these activities are all necessary for viral replication (Brinton, 2002). The NS5 protein has two domains, a C-terminal RNA-dependent RNA polymerase (RdRp) and the N-terminal methyltransferase core, which enables NS5 to exhibit the (nucleoside-2′-O-)-methyltransferase activity required for methylation of the cap
structure at the 5’ end of the RNA genome (Egloff et al., 2002). Furthermore, the NS5 protein has recently been identified as an interferon antagonist due to its ability to inhibit STAT1 phosphorylation in response to interferon and its association with interferon receptor complexes (Best et al., 2005).

It has been demonstrated that low pH plays an essential role in both the entry and the release stages of the flavivirus life cycle (Stadler et al., 1997). During the first stage of the viral life cycle, the virions bind to the surface of the host cell, mediated by the viral surface E protein and with heparan sulfate as the major host cell receptor.

### Table 1. Mammalian tick-borne flavivirus group

Data are adapted from the following references: Calisher & Gould (2003); Gritsun et al. (2003b); Gould & Solomon (2008); Grard et al. (2007).

<table>
<thead>
<tr>
<th>Virus name</th>
<th>Abbreviation</th>
<th>Principal tick vector</th>
<th>Geographical distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tick-borne encephalitis virus (European subtype)</td>
<td>TBEV-Eu</td>
<td>I. ricinus</td>
<td>Central/western Europe, Scandinavia, Korea</td>
</tr>
<tr>
<td>Tick-borne encephalitis virus (Siberian subtype)</td>
<td>TBEV-Sib</td>
<td>I. persulcatus</td>
<td>Russia, Finland</td>
</tr>
<tr>
<td>Tick-borne encephalitis virus (far-eastern subtype)</td>
<td>TBEV-Fe</td>
<td>I. persulcatus</td>
<td>Russia, Far East (China, Japan)</td>
</tr>
<tr>
<td>Louping ill virus</td>
<td>LIV</td>
<td>I. ricinus</td>
<td>UK, Ireland, Norway</td>
</tr>
<tr>
<td>Spanish sheep encephalomyelitis virus</td>
<td>SSEV</td>
<td>I. ricinus</td>
<td>Spain</td>
</tr>
<tr>
<td>Turkish sheep encephalitis virus</td>
<td>TSEV</td>
<td>I. ricinus</td>
<td>Turkey</td>
</tr>
<tr>
<td>Greek goat encephalitis virus</td>
<td>GGEV</td>
<td>I. ricinus</td>
<td>Greece</td>
</tr>
<tr>
<td>Powassan virus</td>
<td>POWV</td>
<td>Ixodes cookei, Ixodes marxi</td>
<td>USA, Canada, far-eastern Russia</td>
</tr>
<tr>
<td>Kadam virus</td>
<td>KADV</td>
<td>Rhipicephalus pravus</td>
<td>Uganda, Saudi Arabia</td>
</tr>
<tr>
<td>Omsk hemorrhagic fever virus</td>
<td>OHFV</td>
<td>Dermacentor reticulatus (Dermacentor marginatus)</td>
<td>Western Siberia</td>
</tr>
<tr>
<td>Kyasanur Forest disease virus</td>
<td>KFDV</td>
<td>Haemaphysalis spinigera (Ixodes spp., Dermacentor spp., Haemaphysalis spp.)</td>
<td>India</td>
</tr>
<tr>
<td>Alkhurma hemorrhagic fever virus</td>
<td>AHFV</td>
<td>Ornithodorus savignyi</td>
<td>Saudi Arabia</td>
</tr>
<tr>
<td>Langat virus</td>
<td>LGTV</td>
<td>Ixodes granulatus</td>
<td>Malaysia, Thailand, Siberia</td>
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<td>Karshi virus</td>
<td>KSIW</td>
<td>Ornithodorus papillipes</td>
<td>Uzbekistan</td>
</tr>
<tr>
<td>Royal Farm virus</td>
<td>RFV</td>
<td>Argas hermanni</td>
<td>Afganistan</td>
</tr>
<tr>
<td>Gadgets Gully virus</td>
<td>GGYV</td>
<td>Ixodes uriae</td>
<td>Macquarie Island (Southern Ocean)</td>
</tr>
</tbody>
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**Fig. 2.** Phylogenetic analysis of tick-borne flaviviruses based upon the NS3 protein, adapted from the report by Grard et al. (2007) (condensed tree; length of horizontal lines is not proportional to genetic distance). Abbreviations for the mammalian tick-borne flaviviruses are detailed in Table 1. Abbreviations for the seabird tick-borne flaviviruses are as follows: SREV, Saumarez Reef virus; MEAV, Meaban virus; TYUV, Tyuleniy virus; CFAV, cell fusing agent virus.
et al. (2003). Work with WNV suggests that the virions are then transported by receptor-mediated endocytosis through clathrin-coated pits into prelysosomal endocytic compartments of the host cell (Chu & Ng, 2004). Recently, it has been demonstrated that the low pH within the endosome causes the protonation of a conserved histidine residue (His323) at the interface between domains I and III of the E protein (Fritz et al., 2008), inducing a change in conformation where the E protein is reorganized from dimers into trimers (Allison et al., 1995b). The fusion peptide is now exposed and fusion of the viral membrane with the endosomal membrane is induced, releasing the viral nucleocapsid into the host cell cytoplasm, where translation of the genome RNA occurs (reviewed by Lindenbach & Rice, 2001). Uncoating of the viral RNA genome occurs and the viral polyprotein is processed to yield individual viral proteins (reviewed by Chambers et al., 1990a). This leads to initiation of viral genome replication, where full-length negative-strand copies of the genome act as templates for the production of new positive-strand RNAs (Chu & Westaway, 1985). During polyprotein synthesis, the surface proteins prM and E are translocated into the lumen of the ER and their amino-termini are released through proteolytic cleavage by host cell signalase (Nowak et al., 1989). The highly basic C protein packages the RNA genome into nucleocapsids on the cytoplasmic side of the ER membrane and, at the same time, assembly of the viral envelope containing prM and E occurs through budding of the nucleocapsid into the ER lumen (Chambers et al., 1990a). This assembly yields non-infectious immature virions, where proteins prM and E are in a heterodimeric association on the viral surface (Lorenz et al., 2002; Elshuber et al., 2003) and are transported through the host secretory pathway. In the acidic vesicles of the late trans-Golgi network, cleavage of prM protein by the host cell protease furin leads to virus maturation (Stadler et al., 1997). This final activation cleavage leads to production of protein M and the reorganization of E protein into fusion-competent homodimers; the infectious mature virions are released from the cell through fusion of the transport vesicles with the host cell plasma membrane (Wengler & Wengler, 1989).

The three main subtypes of TBEV are the European, Siberian and far-eastern subtypes (Ecker et al., 1999), which are all closely related both genetically and antigenically. Studies with European TBEV isolates suggest that TBEV is quite stable under natural ecological conditions and does not undergo significant antigenic variation. Indeed, there is known to be a high degree of antigenic homogeneity between different strains of TBEV (Holzmann et al., 1992). Culturing TBEV in the laboratory is known to affect both the genotypic and the phenotypic characteristics of the virus, and phenotypic characteristics are also thought to change following mammal-to-tick transmission of the virus (Kaluzová et al., 1994; Romanova et al., 2007). Infectious cDNA clones composed of two different strains of TBEV have been demonstrated as a useful experimental system for the specific mutagenesis of TBEV, exhibiting identical biological properties to the parent viruses (Mandl et al., 1997).

TBEV complex viruses cause limited disease in indigenous forest animals but have the potential to emerge as pathogens if they infect introduced species. During the last few thousand years, this group of viruses has evolved and spread westwards throughout Asian and European forests (Gould et al., 2006). TBEV is now endemic in an area ranging from northern China and Japan, through far-eastern Russia to Europe (Dumpis et al., 1999; Hou et al., 1997; Takashima et al., 1997). The European subtype of TBEV is predominantly found throughout Europe and Russia, with Russia also having the Siberian subtype of TBEV. The far-eastern subtype of TBEV is endemic in northern regions of China and is also present in western and south-western China (Lu et al., 2008). This subtype has also been shown to be endemic in Japan (Takashima et al., 1997) and is present in far-eastern Russia.

Phylogenetic studies of a number of TBEV genes over the last few years have all generated trees with a similar pattern (Gould et al., 2004). Studies have been based upon a number of nucleotide sequences, including the E, NS3 and NS5 sequences, and it has been shown that NS3 generates a robust phylogenetic analysis, which is very similar to that obtained from complete sequences (Cook & Holmes, 2006; Grard et al., 2007). Recent studies have shown that the far-eastern and Siberian subtypes are phylogenetically more closely related to each other than to the European subtype, which appears to be more closely related to LIV (Grard et al., 2007) (Fig. 2). LIV is the only tick-borne flavivirus currently found in the UK; it is transmitted by Ixodes ricinus and causes disease primarily in sheep and red grouse, predominantly distributed on the sheep-rearing hillsides of Scotland, England, Wales and Ireland (McGuire et al., 1998). In comparison, TBEV is generally found in forests throughout Europe and Asia (Gritsun et al., 2003a). Unlike TBEV, LIV causes viraemia and fatal encephalomyelitis in domesticated animals when infected ticks feed on them (McGuire et al., 1998). Although LIV is potentially a threat to humans in the UK, natural exposure is rare and there have been very few reports of cases in humans (Gritsun et al., 2003a). The few reported human cases of LIV have mainly been laboratory-acquired (Davidson et al., 1991) and the disease course is similar to that observed with TBEV (Gritsun et al., 2003a). However, although the disease is often subclinical in humans, the meningoencephalitis caused can be severe (Davidson et al., 1991). A disease similar to that caused by LIV has been reported in sheep and goats in other European countries, including Spain, Greece and Turkey (Gritsun et al., 2003a). The viruses causing disease are genetically distinguishable from LIV and have been named according to the country they were first isolated in, for example Spanish sheep encephalomyelitis, Greek goat encephalitis and Turkish sheep encephalitis (Table 1) (Grard et al., 2007). Although domestic animals infected
with TBEV are generally asymptomatic, many studies have demonstrated the presence of circulating antibodies to TBEV in both animal and human populations in countries throughout Europe, including Lithuania, Norway and Sweden (Juceviciene et al., 2005; Csángó et al., 2004; Stjernberg et al., 2008). In Denmark, it was found that 8.7 % of roe deer were seropositive (Skarphédinsson et al., 2005) and a recent study of a small endemic island in south-east Sweden has found human seropositivity to be 2 % (Stjernberg et al., 2008). Similarly, a human study in south-eastern Turkey found circulating TBEV-specific IgG (10.5 %) and IgM (23 %) antibodies, suggesting possible human cases despite there being no reported cases of TBE in Turkey to date. However, these are ELISA-derived data and they have not been confirmed by neutralization tests (Ergunay et al., 2007). Often, there appears to be a correlation between seropositivity in domestic animals, the number of infected ticks and the number of human clinical cases of TBE in a particular region (Juceviciene et al., 2005). A recent study has shown that a significant number of domestic dogs in the Aust-Agder region of southern Norway had circulating antibodies to TBEV, even though TBE had not previously been observed in this region (Csángó et al., 2004). These data correlated with the first cases of human TBE in Norway and provide further evidence that TBEV can be considered an emerging pathogen. Furthermore, strains of the Siberian subtype of TBEV were isolated from Ixodes persulcatus ticks in Finland in 2004, suggesting that I. persulcatus and Siberian TBEV are found significantly further north-west of the previously known range in eastern Europe and Siberia (Jääskeläinen et al., 2006). Indeed, all three known subtypes of TBEV are capable of co-circulating at the same time in the same area, as is currently the situation in Estonia (Golovljova et al., 2004).

It has been shown that nymphs and larvae co-feed together on the same rodent host, and the extent to which this occurs depends upon the patterns of seasonal activity which is influenced by temperature (Randolph et al., 2000). The apparent increase in incidence of TBEV over the last two decades has been attributed to climate change (affecting both tick and rodent population dynamics) in many reports, although in reality, it is likely that a number of factors have simultaneously influenced this (Sumilo et al., 2007). Apart from changing climate conditions, social, political, ecological, economic and demographic factors all appear to play a role in aiding the spread of tick-borne disease (Süss, 2008). These include changes in land usage (such as increased forestation or newly created gardens) and the growing popularity of outdoor pursuits such as hill-walking and fishing. In particular, socio-economic conditions have been shown to have an impact on the incidence of TBE, as people who exist in poverty [for example, through unemployment, or political upheaval (such as the break-up of the Soviet Union)] are less likely to be vaccinated against TBEV and more likely to go foraging for food such as wild fruit and mushrooms in the forest, thus increasing the risk of a tick bite and of contracting TBE (Sumilo et al., 2008a). However, this does not explain the increasing incidence in countries such as Germany, Italy, Finland and Sweden (Süss, 2008). Other factors that may have contributed towards the increase in incidence of TBEV in central and Eastern Europe include an increase in the amount of land cultivated and a decrease in both the use of pesticides and the amount of industrial pollution (Sumilo et al., 2008b). These factors would have had a positive effect on the habitat and rodent host populations for I. ricinus ticks. Changes in hunting practices have also led to an increase in larger mammal populations, such as roe deer and wild boar, providing a greater feeding opportunity for questing ticks (Sumilo et al., 2008b). Alternatively, there has been an improvement in the quality of epidemiological surveillance systems and diagnostics, which may also influence the reporting of TBEV cases (Süss, 2003). Furthermore, in an age when travel is increasingly popular, and previously remote areas of the planet are now more accessible, the risk of a traveller becoming infected through a tick bite is greatly increased.

In a recent study, birds migrating from western Russia and Fennoscandia to Sweden have been shown to carry TBEV-infected I. ricinus ticks (Waldenström et al., 2007). This leads to the possibility that migrating birds may play a role in the dispersal of TBEV-infected ticks, although the importance of birds as a reservoir for TBEV has yet to be determined. Indeed, migratory birds have been suggested as one of the routes by which far-eastern strains have been reported as far west as Latvia and Estonia (Golovljova et al., 2004; Gould & Solomon, 2008). The ecological and phylogenetic characteristics of TBEV and other flaviviruses (both mammalian and seabird-borne) suggest that ticks that feed on both mammals and seabirds may form the evolutionary bridge between these different lineages (Grard et al., 2007).

**Vector ecology**

In western Europe, TBEV is transmitted primarily by the I. ricinus tick (Fig. 3a), whereas the vector for the Siberian and far-Eastern subtypes is I. persulcatus (Gritsun et al., 2003b). I. ricinus, the principal vector for the European subtype of TBEV, is a three-host tick, where each parasitic stage (larva, nymph and adult females) feed for a period of a few days on a different host from a range of species (Gray, 1991) (Fig. 4). Each stage of the life cycle takes approximately 1 year to develop to the next, so the entire life cycle is generally completed in 3 years, although this can vary from 2 to 6 years, depending upon the geographical location (Gray, 1991). If infected with TBEV, the ticks remain infected throughout their life cycle (Gritsun et al., 2003a); nymphs are thought to be the most important stage in the transmission of TBEV as they are more numerous than adults (Süss, 2003). Throughout the majority of its geographical range, I. ricinus becomes active and starts feeding on a range of hosts in spring and early
summer, with ticks being observed on vegetation and animals from late March (Gray, 1991). A study in the UK has shown that the tick will parasitize any mammal or bird that it meets, although adults only feed successfully on larger animals such as deer or livestock (Milne, 1949). Generally, throughout Europe, one major peak of tick activity is seen, where high numbers of larvae and nymphs occur together, peaking around April/May, or as late as July in cooler Scandinavia (Randolph et al., 2000). It is during this peak that there is sufficient transmission to allow TBEV to persist within TBE foci, where coincident feeding by larvae and nymphs on rodent hosts is essential for the maintenance of TBEV transmission (Randolph et al., 1999). The tick is infected with TBEV during feeding; virus replication commences after entry into cells of the midgut wall, and infection of the salivary glands occurs prior to transmission in the tick saliva during the next blood meal (Nuttall et al., 1994). The infected tick can transmit the virus to a vertebrate host during feeding and is also able to pass on the virus to a non-infected tick during co-feeding at the same site on the host. Studies into the transmission of TBEV between co-feeding ticks have shown that vertebrate hosts can play an important role in TBEV transmission in the absence of a detectable level of viraemia (Labuda et al., 1993). Natural hosts that have detectable levels of neutralizing antibodies and no detectable viraemia can still support transmission of virus between infected and uninfected ticks feeding closely (Labuda et al., 1997). The process of virus transmission between ticks during co-feeding is thought to occur via cellular infiltration of tick feeding sites and the migration of cells from these sites (Labuda et al., 1996). Studies have shown that mass co-feeding of larvae alone is also important in the persistent circulation of TBEV, as well as the feeding of infected nymphs alongside infectable larvae (Danielová et al., 2002). Furthermore, the isolation of TBEV from unfed larvae provides evidence for transovarial transmission of TBEV (Danielová et al., 2002). In the UK, it has been shown that *I. ricinus* becomes biologically active at temperatures of 11 °C and above, and that the lower limit of humidity for survival is represented by a relative humidity value of between 70 and 80% (MacLeod, 1935), hence the forests of Europe and Asia provide an ideal tick habitat, with high humidity in the dense undergrowth that prevents desiccation (Gritsun et al., 2003a). Furthermore, it has been shown that both larval–nymph synchrony, where nymphs and larvae co-feed together on the same rodent host, and TBE foci are statistically significantly associated with temperature, characterized by a rapid fall in ground level...
temperatures from August to October (Randolph et al., 2000).

Studies have shown a variable prevalence of infected I. ricinus ticks; up to 1.7% in areas of Lithuania (Han et al., 2005) and as high as 14.3% in a recognized TBEV focus in Switzerland (Casati et al., 2006). Recently, it has been reported that the prevalence of TBEV in ticks removed from humans was significantly higher than in unfed, free-living I. ricinus ticks in the same area, possibly associated with different replication strategies of the virus in tick and mammalian cells (Süss et al., 2006). Furthermore, ticks are capable of maintaining a double infection, being able to transmit both TBEV and Borrelia to humans, as demonstrated by I. persulcatus ticks in Russia (Korenberg, 1994).

TBEV is maintained in cycles involving Ixodid ticks and wild mammalian hosts (Fig. 3b), particularly rodents (Charrel et al., 2004). The rodent host acts as both maintenance and amplifying host and also as a reservoir host (Süss, 2003), and it has been suggested that small mammals may maintain a persistent infection with TBEV throughout the year (Bakhvalova et al., 2006). Although larger animals (such as birds, deer and horses) may act as hosts for ticks, they are not thought to have an important role in virus transmission between ticks (Grisun et al., 2003a). Indeed, studies have shown that a localized absence of deer increases tick feeding on rodents, with the potential to cause tick-borne disease hotspots (Perkins et al., 2006). In western Slovakia, yellow-necked field mice and bank voles comprise around 75% of the rodent population, of which approximately 15% are seropositive for TBEV (Kozuch et al., 1990). A recent study in Korea demonstrated molecular detection of European subtype TBEV genes in 20% of wild rodents sampled, and in 10% of Ixodes nipponensis ticks sampled (Kim et al., 2008). This was the first report of TBEV in Korea, where the number of patients presenting with encephalitis of unknown origin was seen to be increasing annually. It is interesting that it was a European rather than a far-eastern strain of TBEV that was detected in this study, as one would have expected a far-eastern strain similar to those found in Japan and China. Furthermore, I. nipponensis is not the usual tick vector for European TBEV and may provide further indications that TBEV can be considered an emerging pathogen. Similarly, although the predominant vector in China is I. persulcatus, a number of additional vectors are thought to be involved in the transmission of TBEV, including Ixodes ovatus in Yunnan province (south-western China), a region where I. persulcatus is not detected (Lu et al., 2008).

Pathogenesis

The majority of TBEV infections occur through a tick bite, although a small number of infections occur through consuming infected unpasteurized milk (Dumpsis et al., 1999). Following infection with tick-borne flaviviruses (either naturally or experimentally via intradermal or subcutaneous routes), the virus is known to first replicate at the site of inoculation and then in the lymph nodes that drain the inoculation site (Albrecht, 1968). TBEV has been found in the Langerhans cells of the skin before reaching the regional lymphatic nodules via the lymphatic system (Haglund & Günther, 2003). Virus replication in the draining lymph nodes is followed by development of plasma viraemia (Málková & Frańková, 1959). During the viraemic phase, many extra-neural tissues are infected and the release of virus from these tissues enables the viraemia to continue for several days (Albrecht, 1968; Monath & Heinz, 1996). Haematogenic spread allows different organs to be infected, especially the reticulo-endothelial system (spleen, liver and bone marrow) (Haglund & Günther, 2003), and it is during this phase that the virus also crosses the blood–brain barrier to invade the central nervous system (CNS), where viral replication causes inflammation, lysis and cellular dysfunction (Dumpsis et al., 1999). Neuroinvasian of TBEV in humans has been well reported, although the mechanisms by which an acute, non-fatal febrile infection develops into a severe, possibly fatal CNS disease are not clearly understood (Toporkova et al., 2008).

There are a number of animal models regularly employed to examine the neuropathogenicity of TBEV; the mouse is the most commonly used model as it is susceptible to TBEV-induced disease, unlike other wild and domestic animals (Mandl, 2005). Wild-type strains of TBEV are generally neuropathogenic when experimentally inoculated into mice (intracranially or peripherally) usually resulting in a lethal infection (Mandl, 2005), although this is very much dependent upon the age of the mice (Andzhaparidze et al., 1978). An apparent feature of TBEV is the ability to cause persistent infections in experimental animals and humans (Monath & Heinz, 1996), and a number of animal models have been used to demonstrate degenerative changes in the CNS following infection with TBEV. Intracranial inoculation of hamsters with the Soph-K strain of TBEV induced clinical disease in 14% of animals, although pathological lesions characterized as meningoencephalitis were found in the CNS of all the animals (Andzhaparidze et al., 1978). Histological examination 45 days post-inoculation demonstrated signs of neuronophagy and marked glial proliferation. In the same study, subcutaneous inoculation of hamsters induced meningoencephalitis in the 4% of animals that were exhibiting clinical disease, although lesions in the brain were observed in the majority of animals, including perivascular infiltration and encephalitis ‘granulomas’. In an earlier study, when monkeys were infected intranasally or intracerebrally with European TBEV, they were shown to develop chronic encephalitis with degenerative spongiform lesions and astrocytic proliferation (Zlontnik et al., 1976). Furthermore, monkeys inoculated intracerebrally with the Soph-K strain of TBEV showed an asymptomatic infection with subacute disseminated meningoencephalitis, with a progradient course for the 3 months of observation; this chronic infection provides a model of progressive degener-
Host immune response

Shortly after the onset of neurological symptoms in humans, the mononuclear cells in the cerebrospinal fluid (CSF) are predominantly composed of CD4+ T lymphocytes and less numerous CD8+ T lymphocytes, with limited natural killer cells and B-lymphocytes (Holub et al., 2002). Levels of TBEV-specific antibodies have been shown to increase in both serum and CSF; maximum IgM levels were seen during early disease and persisted after 6 weeks, whereas peak IgG levels were observed in late convalescent samples (around 6 weeks) (Günther et al., 1997). However, although IgM antibodies can be detected for a few months after infection, IgG antibodies persist for a lifetime and provide a level of immunity that prevents reinfection (Holzmann, 2003).

Recent work has investigated the role of viral proteins in the immune response to TBEV infection. The NS5 protein of TBEV has been shown to act as an interferon antagonist and is able to inhibit interferon-stimulated JAK–STAT signalling by blocking the phosphorylation of STAT1, thus inhibiting the expression of antiviral genes (Best et al., 2005; Werme et al., 2008). However, there are limited data on the role of cytokines and chemokines in the pathogenesis of TBEV. Recently, a number of studies have investigated the level of certain cytokines and chemokines in the serum and CSF of patients with TBE. A study in Russia found that on admission to hospital, TBE patients had elevated serum levels of tumour necrosis factor (TNF)-z, interleukin (IL)-1z and IL-6, where IL-1z and TNF-z were acting synergistically to initiate the cascade of inflammatory mediators by targeting the endothelium; levels of these cytokines then declined during the first week of hospitalization, which was accompanied by an increase in levels of IL-10 (an inhibitor of cytokine synthesis) (Atrasheuskaya et al., 2003). Another study found that during TBEV infection, the concentration of CXCL10 was higher in CSF than in serum, suggesting a role in the recruitment of CXCR3-expressing T cells into the CSF (Lepej et al., 2007). Similarly, another study found that the concentration of CCL5 (another lymphocyte attractant) was increased in the CSF but not the serum of TBE patients, and that this increase was sustained after the disappearance of clinical symptoms (Grygorczuk et al., 2006a). Conversely, it has been reported that although synthesis of CCL3 was increased during TBE infection, its concentration was much lower in CSF than in serum, suggesting that its major role is not as a chemoattractant of leukocytes into the CNS (Grygorczuk et al., 2006b).

Clinical manifestations

The clinical manifestations in human cases have been well-documented and there is a range of symptoms that can be observed.

Although the far-eastern subtype of TBEV causes a monophasic course of illness, infection with a western European subtype usually produces a biphasic course of illness (Dumpis et al., 1999; Gritsun et al., 2003a). The incubation period is generally 7–14 days and during a typical biphasic infection, symptoms during the initial short febrile period can include fatigue, headache and pain in the neck, shoulders and lower back, accompanied by high fever and vomiting (Gritsun et al., 2003a). This is often followed by an asymptomatic period lasting 2–10 days and if the disease progresses to neurological involvement, this leads to the second phase, characterized by acute CNS symptoms with a high fever. CNS infection can manifest in the meninges (where inflammation causes meningitis), the brain parenchyma (to cause encephalitis), the spinal cord (myelitis), the nerve roots (radiculitis) or indeed any combination of these.

Acute TBE is characterized by encephalitic symptoms in 45–56 % of patients (Haglund & Günther, 2003). Symptoms range from mild meningitis to severe meningoencephalomyelitis, which is characterized by muscular weakness (paresis) which develops 5–10 days after remission of the fever. Severely affected patients may demonstrate altered consciousness and a poliomyelitis-like
symptom that may lead to long-term disability (Dumpis et al., 1999; Kleiter et al., 2007; Gritsun et al., 2003a). The acute febrile period of illness correlates with the presence of viraemia. Following the asymptomatic phase, the second stage of illness correlates with virus invasion into the CNS, where viral replication is associated with inflammation, lysis and dysfunction of the cells (Dumpis et al., 1999). Although magnetic resonance imaging (MRI) is usually normal, abnormalities have been shown, including pronounced bilateral lesions in the thalamus, cerebral peduncles and the left caudate nucleus (Lorenzl et al., 1996). However, it has been shown that the abnormalities detected in TBE patients depend upon the MRI technique used, as T2-weighted and turbo FLAIR images demonstrated more abnormalities than T1-weighted images (Marjelund et al., 2004).

A study of TBEV patients in Germany reported that the average incubation period between tick bite and onset of symptoms was 11 days (with a range of 4–28 days) (Kaiser, 1999). This study showed that 74% of patients experienced a biphasic course of illness, typically with the first stage lasting between 1 and 7 days, and consisting of fever, headache, occasional malaise and upper respiratory and/or abdominal symptoms. The intermediate asymptomatic phase lasted between 3 and 21 days, followed by a prodromal period which was reported less often in patients with meningoencephalomyelitis (inflammation of the brain and spinal cord) than in patients with meningoencephalitis (inflammation of the brain and meninges) or isolated meningitis. Of the 656 patients, 49% presented with meningitis, 41% with meningoencephalitis and 10% with meningoencephalomyelitis. In patients with meningoencephalomyelitis, the most common feature was flaccid paresis of the extremities, whereas this was less common with meningoencephalitis, and was usually transitory. Similarly, paresis of the cranial nerves was more frequent and severe in patients with meningoencephalomyelitis. Eight patients (1.2%) were ventilated until death (1–52 weeks after onset of disease). This study also showed that the severity of illness increased with the age of the patient. An earlier study in Sweden reported similar findings, with spinal nerve paralysis observed in 13% of patients; only 60% of patients were considered to be recovered after 1 year (Günther et al., 1997).

A chronic form of TBE has been observed in patients from Siberia and far-eastern Russia and is thought to be associated with the Siberian subtype of TBEV (reviewed by Gritsun et al., 2003a). There are two forms of chronic TBE, the first being long-term sequelae of any of the acute forms of TBE, where the development of neurological symptoms may take years following the bite from the infected tick. Clinical symptoms include Kozshevnikov’s epilepsy, progressive neuritis of the shoulder plexus, lateral and dispersed sclerosis, a Parkinson’s-like disease and progressive muscle atrophy. Often the physical deterioration is accompanied by mental deterioration and even death. A second chronic form of TBE is associated with hyperkinesias and epileptoid syndrome. Hyperkinesia occurs frequently and may arise during the acute phase of TBE or persist as Kozshevnikov’s epilepsy.

The incidence of the different forms of TBE is variable according to the region (reviewed by Gritsun et al., 2003a). In Siberia, approximately 80% of TBE cases present with a fever but without neurological sequelae. Paralytic forms are observed in approximately 7–8% of cases and Kozshevnikov’s epilepsy in about 4–5% of patients. In general, the case fatality rate is approximately 1–2% following European subtype infection, but can be as high as 20–40% following infection with a far-eastern subtype (Mandl, 2005). Infection with the Siberian subtype produces a mortality rate of no more than 2–3% (Atrasheuskaya et al., 2003). However, it is possible that the high mortality figures for the far-eastern subtype may be due to the lack of detection of mild cases. A study of 62 cases of TBE in an area of Russia where the Siberian and far-eastern subtypes of TBEV co-exist has shown a large variation in the clinical symptoms observed, ranging from unapparent to severe (Pogodina, 2005). Hence, an increase in detection of milder cases would lead to an overall decrease in mortality rate figures.

In comparison with humans, domestic animals infected with TBEV are generally asymptomatic. Indeed, recent data suggest that wild small mammals are able to maintain TBE as a persistent infection throughout the year (Bakhvalova et al., 2006).

**Diagnosis and treatment**

The clinical symptoms observed with TBE are shared with other neurological disorders such as herpes encephalitis; therefore, differential diagnosis of a TBEV infection must be established in the laboratory (Holzmann, 2003). Diagnosis of infection with TBEV is now possible with a variety of techniques, from both ante-mortem (serum/CSF) and post-mortem (tissue) samples. Along with virus isolation, these techniques include RT-PCR, which is able to detect viral RNA in serum and CSF prior to the appearance of antibodies (Saksida et al., 2005), multiplex PCR to differentiate between TBEV subtypes (Růžek et al., 2007) and quantitative PCR on ante-mortem CSF samples (Schwaiger & Cassinotti, 2003). Most patients present at the hospital during the non-viraemic second phase of disease, when virus has been cleared from the blood and neurological symptoms have commenced (Holzmann, 2003). Development of the humoral immune response enables diagnosis of TBE by ELISA detection of antibodies in the blood, such as TBEV-specific IgM and IgG antibodies (Holzmann, 2003). It has been shown that between 0 and 6 days after onset of encephalitic symptoms, TBEV-specific IgM activity increased in serum, declining after 6 weeks (Günther et al., 1997). In CSF, IgM activity peaked between 9 days and 6 weeks after onset. In comparison, maximum IgG activity in both serum and CSF was in 6 week samples. In countries such as Japan,
human cases of TBE may have been underreported due to the cross-reactivity between TBEV and JEV in serological assays and the similarity of clinical symptoms that are observed with each infection (Takashima et al., 1997).

There is no curative therapy for TBE, so supportive treatment includes paracetamol, aspirin and other non-steroidal anti-inflammatory drugs. In severe cases, some clinicians administer corticosteroids, although their use has not been validated. Patients with severe CNS symptoms have to be closely monitored as coma or neuromuscular paralysis may develop rapidly, in which case intubation and ventilation is necessary (Dumpis et al., 1999).

### Vaccination and prevention

Active vaccination is the most effective method for preventing TBE, with modern vaccines shown to be safe and between 95 and 99% effective (Heinz et al., 2007). Currently, there are at least four vaccines available against TBEV infection, which are manufactured from purified, formaldehyde-inactivated virus. These include: Austrian ‘FMSE-Immun 0.5 ml’ and ‘FSME-Immun 0.25 ml Junior’, German ‘Encepur Adult and Children’ and two Russian vaccines manufactured in Moscow and Tomsk (Leonova et al., 2007). The European vaccines are produced using the Neudorf (Austria) and K23 (Germany) strains of the European subtype (Charrel et al., 2004), whereas the Russian vaccines are produced from the far-eastern strains 205 and Sofjin (Leonova et al., 2007).

An annual TBE vaccination campaign was introduced in Austria in 1981. Despite once having the highest incidence rate in Europe (up to 700 hospitalised cases annually), this campaign caused a steady decline in the number of cases of TBE (Süss, 2008). However, with the exception of Austria, vaccination campaigns have had varying degrees of success, since the vaccines are relatively expensive and must have repeated administrations in order to maintain protective immunity (Juceviciene et al., 2005). Post-exposure injection with specific immunoglobulin, when given within 96 h of exposure, has been shown to prevent disease in approximately 60% of patients (Dumpis et al., 1999). However, post-exposure prophylaxis should be discouraged, as it has been suggested that the administration of post-exposure passive immunization may actually exacerbate the disease through antibody-dependent enhancement of infection (Arras et al., 1996; Phillpotts et al., 1985).

Studies in mice have demonstrated a high level of cross-protection between European and far-eastern subtypes of TBEV, where immunization with the European subtype vaccine protected the mice against infection with far-eastern strains (Holzmann et al., 1992). Furthermore, a recent study in human volunteers has shown that following vaccination with Encepur Adult (based upon the European subtype), they were able to produce a humoral response towards strains of the far-eastern TBEV subtype (Leonova et al., 2007). This neutralization assay data demonstrated that the human sera contained neutralizing antibodies capable of neutralizing far-Eastern TBEV strains, suggesting that this vaccine may offer protection in humans against a viral infection caused by a far-eastern strain of TBEV.

A recent study in Lithuania demonstrated that antibodies to TBEV were found most often in people who frequently spent time in the countryside or who had consumed unpasteurized goats’ milk (Juceviciene et al., 2002) and there have been many reports of the transmission of TBEV to humans through the consumption of unpasteurized goats’ milk (causing ‘biphasic milk fever’). This oral route of infection is possible, since TBEV has been shown to retain infectivity within the low pH of gastric juices (Pogodina, 1958). Similarly, the consumption of unpasteurized cows’ milk is common in a number of countries, including Lithuania, and has been suggested as a minor route of infection to humans (Juceviciene et al., 2005). Therefore, campaigns encouraging people to pasteurize all milk, particularly goats’ milk, before consumption may help to reduce the risk of exposure.

Vaccination of those individuals who are most at risk would therefore be advantageous, although the risk of infection is dependent upon factors such as location, season and activity. As recent data from Latvia confirm, it is clear that people who visit forests (for work, food collection or leisure) are four to five times more likely to encounter ticks than people who do not visit forests (Šumilo et al., 2008a). With the growing popularity for people to spend their leisure time in the countryside, further measures alongside vaccination could be encouraged in order to prevent infection through a tick bite. These include the use of tick repellents in combination with the wearing of appropriate clothing (for example, long trousers) and avoidance of the tick habitat if possible (Dumpis et al., 1999), although a recent study has shown that tick repellents are only 20% effective (Vázquez et al., 2008). Indeed, a recent study has suggested that risk avoidance through changing human behaviour (independent of the seasonal changes in tick activity) has led to a decrease in incidence of TBE infection in a number of Baltic countries in recent years (Šumilo et al., 2008a).

Similarly, vaccination of people travelling to areas known to be endemic for TBEV is advisable.

### The current situation

As an emerging zoonosis, there is currently increasing interest in TBEV. In particular, this has prompted the identification of particular regions of the genome that may influence phenotypic characteristics of the virus. Recently, certain mutations that influence the neuroinvasive capacity of the virus in mice have been identified (Růžek et al., 2008). This has suggested that non-virulent variants of TBEV, which are highly adapted to ticks yet sustain human population immunity by inducing subclinical infections, are found in the natural environment. Furthermore, these
authors suggest that virulent and attenuated viruses may co-exist as quasispecies in the same TBEV population and that conversion of neurovirulence during tick/mammal adaptation of the virus is mediated by selection from this quasispecies population rather than random mutagenesis.

There is mounting speculation that TBEV has the potential to move further westward towards the UK. Predictive models suggest that the UK can potentially be considered as an emerging disease ‘hotspot’, with the likelihood that new emerging infectious diseases are likely to appear (Jones et al., 2008). Increased temperatures have already allowed the limit of I. ricinus to extend north- and westwards, thus fuelling the prediction that TBEV may also extend to previously unaffected areas (Randolph & Rogers, 2000). Indeed, in South Korea it has been shown that western TBEV is in a species of tick that is not normally associated with this virus subtype, and the detection of I. persulcatus ticks in Finland suggests a possible extension to the previously known geographical range of this vector. Conversely, in some areas, climate change may actually disrupt the conditions required for enzootic cycles of TBEV (Randolph & Rogers, 2000). However, in an age of climate change, the increasing popularity of outdoor pursuits and changes in land usage, this is a risk that cannot be ignored. There are clearly a number of factors that may influence the incidence and extent of TBEV throughout Europe and will continue to do so in the years to come.

Acknowledgements

This work was funded by the Department for Environment, Food and Rural Affairs (Defra) grants SE4106 and SCO213. T. S. is an MRC senior clinical fellow.

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