Tick-borne encephalitis virus in ticks in Finland, Russian Karelia and Buryatia

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Tick-borne encephalitis (TBE) is a central nervous system infection caused by a flavivirus [tick-borne encephalitis virus (TBEV)], transmitted by Ixodes ticks and endemic in a large region in Eurasia. We collected 2411 ticks from Finland and Russia in 2003–2008, screened them for TBEV by RT-PCR and isolated and analysed eight strains belonging to all three TBEV subtypes; in addition, we obtained two European-subtype strains from human serum samples. TBEV RNA prevalence in unengorged ticks was approximately 1 % both in the northernmost TBE-endemic areas of Europe in Finland and Russian Karelia, and in Siberia in Buryatia. In Finland, both Ixodes ricinus and Ixodes persulcatus ticks were found from distinct areas and, in Russian Karelia, were overlapping in the same study site. TBEV E and NS3 gene sequences obtained showed a variability of 0–4 % within European-subtype strains, 2–9 % for Siberian-subtype strains and 3–13 % for Far Eastern-subtype strains.

Tick-borne encephalitis (TBE) is a central nervous system disease caused by a flavivirus, tick-borne encephalitis virus (TBEV). There are three subtypes of the virus: European (TBEV-Eur), Siberian (TBEV-Sib) and Far Eastern (TBEV-FE) (Ecker et al., 1999; Thiel et al., 2005). The principal vector for TBEV-Eur is the sheep tick Ixodes ricinus, which is distributed in continental Europe and the British Isles, excluding northernmost Fennoscandia and Russia. TBEV-Sib and TBEV-FE subtypes are carried by the taiga tick Ixodes persulcatus, which is distributed from eastern Europe through Siberia to China and Japan and also on the western coast of Finland (Jääskeläinen et al., 2006). TBEV occurs typically in endemic foci within the distribution area of its vectors (Randolph et al., 2000; Lindquist & Vapalahti, 2008).

The symptoms of TBEV infection in humans vary from none to severe meningoencephalitis. About one-third of patients have been reported to have long-term sequelae [e.g. 36 % by Haglund et al. (1996)]. The case fatality rate in Europe is <2 % (Lindquist & Vapalahti, 2008). The TBEV-Sib subtype seems to lead to a chronic TBEV infection more often than TBEV-Eur (Gritsun et al., 2003a, b; Charrel et al., 2004; Poponnikova, 2006; Lindquist & Vapalahti, 2008) and the reported case fatality rates are 2–8 % (Gritsun et al., 2003a; Charrel et al., 2004; Lindquist & Vapalahti, 2008; Mansfield et al., 2009). TBEV-FE infection is the most severe and leads to the focal form more often than the other subtypes (Gritsun et al., 2003a).

The GenBank/EMBL/DDBJ accession numbers for the partial TBEV 5′NCR, E, NS3 and NS5 sequences determined in this study are HM051160–HM051190 (see Table 2).

Two supplementary tables showing details of patient serum virus-isolation experiments and virus strains used in phylogenetic analyses are available with the online version of this paper.
Between 1990 and 2007, a total of 157,584 TBE cases were reported (Süss, 2008). This is a mean of 8755 year⁻¹, of which 2805 are from Europe excluding Russia. In Russia, about 58 million people live in the TBE-endemic area extending throughout the whole country (Süss, 2008). In addition, TBE is endemic in parts of China, Japan, Korea and Mongolia.

Finland is located in the northernmost limit of the TBE-endemic area in Europe, and TBEV has been endemic in certain foci either in the Åland Islands or the archipelago of Turku (Tuomi & Brummer-Korvenkontio, 1965; Han et al., 2001), in Isosaari in Helsinki archipelago (Han et al., 2001) and in Kokkola archipelago further north (Tuomi & Brummer-Korvenkontio, 1965; Jääskeläinen et al., 2006), or by Lake Saimaa in south-eastern Finland (Tuomi & Brummer-Korvenkontio, 1965) (Fig. 1a). The two Ixodes species most relevant for TBEV transmission and epidemiology, I. ricinus and I. persulcatus, have their distribution areas overlapping in the Baltics and in north-western Russia. Both tick species have been found in Finland, I. persulcatus in an isolated TBE focus on the western coast (Jääskeläinen et al., 2006). The first TBEV isolations from Finland were made from Kumlinge Island in Åland in 1959 and from Joutseno in south-eastern Finland in 1960 (Brummer-Korvenkontio et al., 1973).

Between 2003 and 2008, we collected 2411 ticks from (i) the known TBE-endemic foci in Finland, (ii) the transition zone of the two tick species, Russian Karelia, and (iii) Buryatia at Lake Baikal in the middle of the range of I. persulcatus (Fig. 1a; Table 1) in an attempt to study further the tick species and the prevalence and molecular epidemiology of TBEV.

We collected 494 ticks from two republics of Russia: Karelia in north-western Russia, where, in our panel, five ticks were I. ricinus and 193 I. persulcatus; and Buryatia in eastern Siberia, where the ticks were I. persulcatus. From Finland, we collected 1917 ticks. The samples studied from the Åland Islands and Turku archipelago, Isosaari and Lappeenranta were I. ricinus; in Närpio, central coast of the Gulf of Bothnia, we found exclusively I. persulcatus. These results extend previous findings concerning the western range of I. persulcatus and suggest that there is a zone of I. persulcatus by the northern coastline of the Baltic Sea, including at least Kokkola archipelago (Jääskeläinen et al., 2006; Alekseev et al., 2007) and Närpio approximately 150 km south (Fig. 1a).

The distribution of the two tick species directly reflects the epidemiology of different TBEV subtypes. Efficient circulation of TBEV-Eur has been shown to depend on the co-feeding of I. ricinus nymphs and larvae, which in turn is affected by climatic factors (Randolph et al., 2000). However, it is possible that TBEV-Sib and TBEV-FE, the radiation of which has happened considerably earlier than that of TBEV-Eur (Lindquist & Vapalahti, 2008), may be less sensitive to climate requirements for co-feeding and thus could be found more widely within the I. persulcatus range. The ticks from Kumlinge, Isosaari, Lappeenranta and Turku archipelago were pooled (two to ten ticks per pool) and the ticks from Närpioi, Russian Karelia and Buryatia were handled individually. We isolated RNA from ticks or tick pools by using TriPure Isolation Reagent (Roche Diagnostic Corporation) according to the manufacturer’s instructions, and screened the samples for TBEV RNA by NS5 RT-PCR (Puchhammer-Stöckl et al., 1995) (Kumlinge), by the modified version (Jääskeläinen et al., 2006) (Lappeenranta, Isosaari, Turku archipelago, Närpioi, Russian Karelia) or by 5’ non-coding region (NCR) RT-PCR (Schrader & Süss, 1999) (Isosaari, Lappeenranta, Russian Karelia, Buryatia). Positive samples were subjected to virus-isolation experiments in suckling mice. From the mouse brains, we isolated RNA by using TriPure and performed E RT-PCR (Jääskeläinen et al., 2006; Melik et al., 2007) and NS3 RT-PCR (Billoir et al., 2000; Grard et al., 2007). A similar isolation approach was done for nine Finnish TBE patient samples for which there was an early-phase TBEV antibody-negative serum sample available, drawn 10–28 days before a TBEV IgM-positive serum. Two human samples were positive in virus isolation (see Supplementary Table S1, available in JGV Online). The RT-PCR-positive amplicons and partial E and NS3 genes from the virus isolations were sequenced (GenBank accession numbers are given in Table 2) and used for phylogenetic analysis (Fig. 1b, c).

We studied ticks from a wide geographical range by similar methods and found the prevalence of TBEV in field-collected ticks to be around 1%, independent of the year of tick sampling, region, tick species, TBEV subtype or RT-PCR method, except for the I. ricinus panel of 1039 ticks from Turku archipelago in 2007, where the TBEV prevalence was only 0.1%, and Närpioi, where all 36 ticks were TBEV-negative (Table 1). TBEV prevalences of 0.2–2.0% have usually been reported in questing ticks in TBE-endemic areas in Europe [e.g. 0.2% in Åland and 0.7% in Isosaari in 1996–1997 (Han et al., 2001); 1% in Kokkola archipelago, Finland, in 2004 (Jääskeläinen et al., 2006); 0.5–2.0% in Bavaria, Germany (Süss et al., 2006)]. However, the prevalence of TBEV in ticks even at the same site varies in different years, e.g. in Latvia, TBEV prevalence varied between 1.7 and 26.6% in I. ricinus and between 0 and 37.3% in I. persulcatus ticks in 1995–2002 (Bormane et al., 2004). In the Latvian study, the TBEV-detection method was ELISA and not RT-PCR, thus comparison of the results in prevalence is difficult. Furthermore, that study included not only questing ticks, but also ticks collected from humans and, as described in several German studies, TBEV prevalence in engorged I. ricinus ticks collected from humans is higher than in questing ones (Süss et al., 2004, 2006; Klaus et al., 2010).

The low prevalence in Turku archipelago compared with other TBE-endemic areas may have been influenced by different selection of the tick-collection sites: elsewhere, we concentrated on foci where TBE patients had probably contracted the tick bite, but in Turku archipelago, tick collection was more random and might have included
Fig. 1. (a) Map of sample-collection sites. Yellow, TBE-endemic areas; pink dotted line, *I. ricinus* distribution; green solid line, *I. persulcatus* distribution; ▲, tick-sampling sites (Russia: between Lake Baikal and Ulan-Ude in the Republic of Buryatia, and north of Petrozavodsk in the Republic of Karelia; Finland: Isosaari, Åland and Turku archipelagos, Närpiö and Lappeenranta (LPR)). (b, c) Phylogenetic trees of partial TBEV E (b; 818 nt) and NS3 (c; 604 nt) gene sequences. The trees were reconstructed by using the Bayesian Markov chain Monte Carlo method in BEAST (http://beast.bio.ed.ac.uk/). Maximum clade credibility trees with an arbitrary root are shown with mean branch lengths, and Bayesian posterior probabilities are given at nodes when >0.7. Bars, 0.07 (b) or 0.03 (c) substitutions per site. Country of origin and year of isolation are indicated. Strains described in this study are highlighted in red. Fin-human-2007 and Fin-human-2008 represent TBEV isolations from patient serum samples; both individuals had visited the southern and/or south-western archipelagos of Finland during the incubation period.
islands where TBEV is not circulating. As the transmission cycle of TBEV-Eur is fragile, microclimatic conditions may affect its survival in nature (Randolph et al., 2000) and it is possible that the risk of TBE is not distributed equally within the archipelago.

We compared an 818 nt sequence from the E gene (Fig. 1b) and a 604 nt sequence from the NS3 gene (Fig. 1c) from the TBEV-positive samples and virus isolates obtained in this study with 81 E and 32 NS3 gene sequences available in GenBank (see Supplementary Table S2, available in JGV Online). The nucleotide and amino acid variation within the subtypes based on the strains described here and other partial E and NS3 sequences available was 0–4 and 0–2 % for TBEV-Eur, 2–9 and 0–3 % for TBEV-Sib, and 3–13 and 0–3 % for TBEV-FE strains, respectively.

The TBEV strains from Åland (Kumlinge), Isosaari, Turku archipelago (Korppoo) and Finnish patients belonged to the TBEV-Eur subtype. The partial E and NS3 gene sequences showed that, at the nucleotide level, these European strains were at least 96 % identical to other TBEV-Eur strains. Geographical clustering among the European-subtype TBEV was not observed on any larger scale (Fig. 1b, c). This is in contrast to many zoonoses distributed by terrestrial mammals, e.g. Puumala virus, carried by bank voles (Asikainen et al., 2000), and classical rabies virus, carried by terrestrial carnivores (Bourhy et al., 1999; Metlin et al., 2007), for which strains have been shown to form geographically distinct phylogenetic groups or where geographical barriers limit spreading of strains. TBEV-Eur strains from the same region rarely had most recent common ancestors, e.g. strains from Finland seemed

<table>
<thead>
<tr>
<th>Panel</th>
<th>No. ticks</th>
<th>No. tick pools</th>
<th>Tick species</th>
<th>No. positive* by:</th>
<th>TBEV subtype</th>
<th>Prevalence (%)*</th>
</tr>
</thead>
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<tr>
<td>Kumlinge 2003</td>
<td>454</td>
<td>46</td>
<td>I. ricinus</td>
<td>4</td>
<td>ND</td>
<td>3</td>
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<td>Isosaari 2005</td>
<td>96</td>
<td>11</td>
<td>I. ricinus</td>
<td>1</td>
<td>2</td>
<td>(3)</td>
</tr>
<tr>
<td>Buryatia 2005</td>
<td>296</td>
<td>NA</td>
<td>I. persulcatus</td>
<td>ND</td>
<td>2</td>
<td>(3)</td>
</tr>
<tr>
<td>Karelia 2006</td>
<td>198</td>
<td>NA</td>
<td>I. persulcatus, I. ricinus</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Turku archipelago 2007</td>
<td>1039</td>
<td>315</td>
<td>I. ricinus</td>
<td>1</td>
<td>ND</td>
<td>1</td>
</tr>
<tr>
<td>Lappenranta 2005</td>
<td>292</td>
<td>29</td>
<td>I. ricinus</td>
<td>0 (2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Närpioö 2008</td>
<td>36</td>
<td>NA</td>
<td>I. persulcatus</td>
<td>0</td>
<td>ND</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Number of TBEV RT-PCR-positive ticks is shown in parentheses; however, we could not confirm all of them by sequencing or isolation.

Table 2. GenBank accession numbers of the nucleotide sequences obtained in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Year</th>
<th>Partial 5’NCR</th>
<th>Partial E</th>
<th>Partial NS3</th>
<th>Partial NS5</th>
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<td>Kumlinge-24</td>
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<td>HM051166</td>
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<td>Kumlinge-38</td>
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<td>HM051162</td>
<td>HM051167</td>
<td>HM051188</td>
<td>HM051177</td>
</tr>
<tr>
<td>Kumlinge-39</td>
<td>2003</td>
<td>HM051168</td>
<td>HM051168</td>
<td>HM051174</td>
<td>HM051178</td>
</tr>
<tr>
<td>Kokkola-84</td>
<td>2003</td>
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<td>HM051186</td>
<td>HM051305+</td>
<td>HM051178</td>
</tr>
<tr>
<td>Kokkola-102</td>
<td>2004</td>
<td>DQ451295*</td>
<td>HM051187</td>
<td>HM051305+</td>
<td>HM051180</td>
</tr>
<tr>
<td>Isosaari-5</td>
<td>2005</td>
<td>HM051169</td>
<td>HM051190</td>
<td>HM051169</td>
<td>HM051179</td>
</tr>
<tr>
<td>Buryatia-169</td>
<td>2005</td>
<td>HM051165</td>
<td>HM051175</td>
<td>HM051179</td>
<td>HM051179</td>
</tr>
<tr>
<td>Buryatia-171</td>
<td>2005</td>
<td>HM051164</td>
<td>HM051165</td>
<td>HM051175</td>
<td>HM051189</td>
</tr>
<tr>
<td>Karelia-94</td>
<td>2006</td>
<td>HM051161</td>
<td>HM051173</td>
<td>HM051184</td>
<td>HM051176</td>
</tr>
<tr>
<td>Karelia-108</td>
<td>2006</td>
<td>HM051160</td>
<td>HM051174</td>
<td>HM051185</td>
<td>HM051180</td>
</tr>
<tr>
<td>Korppoo-259</td>
<td>2007</td>
<td>HM051163</td>
<td>HM051170</td>
<td>HM051181</td>
<td>HM051180</td>
</tr>
<tr>
<td>FinHuman-2007</td>
<td>2007</td>
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<td>HM051171</td>
<td>HM051182</td>
<td>HM051183</td>
</tr>
<tr>
<td>FinHuman-2008</td>
<td>2008</td>
<td>HM051172</td>
<td>HM051172</td>
<td>HM051183</td>
<td>HM051183</td>
</tr>
</tbody>
</table>

*For Kokkola-84 and Kokkola-102, the NS3 sequences were obtained in this study; the E and NS5 sequences were already available in GenBank.
to be distributed more or less randomly within the TBEV-Eur cluster. TBEV-Eur has recently been found even in Korea (Kim et al., 2008, 2009). The limited genetic variation and lack of genetic clustering might indicate a recent spread of the virus and possibly a role of migratory birds in dispersing TBEV strains or TBEV-infected ticks, a phenomenon that has been demonstrated e.g. in blackbirds and passerine birds (Ernek et al., 1968; Brummer-Korvenkontio et al., 1973; Waldenström et al., 2007). However, in the E gene sequences obtained, our strains from Kumlinge and Korppoo and a strain from an I. ricinus pool from Kumlinge from 1959 (Brummer-Korvenkontio et al., 1973) were 99–100 % identical to each other, and Isoasari-5 from 2005 was identical to a 150 nt stretch of the NS5 gene available from an I. ricinus pool from the same island from 1996 (Han et al., 2001). Both Kumlinge and Korppoo strains are from islands, thus mammals are not likely to have distributed the virus strains, and one option left could be birds. However, as the Kumlinge strains have not changed more during decades, or the Isoasari strains within a decade, birds do not seem to play a major role in maintenance or further distribution of the TBE foci in this region; if they did, one might expect more variation among the isolated strains. Thus we speculate that migrating birds have probably had a role in establishing the TBE foci, which however are maintained independently without the need for introduction of new virus strains each year.

The province of Karelia is in the transition zone of the two tick species, I. ricinus and I. persulcatus. On the Finnish side of Karelia, there is an endemic TBE focus from where human cases are frequently reported in Lappeenranta, and a TBEV-Eur strain (Joutseno) was isolated from the region in 1960 (Brummer-Korvenkontio et al., 1973). On the Russian side in the Republic of Karelia, the incidence of TBE has been around 10 cases per 100 000 inhabitants year$^{-1}$ in 2004–2008 (EpiNorth, 2010). Two I. persulcatus ticks from Russian Karelia were TBEV-positive and we isolated the TBEV-Sib strains Karelia-94 and Karelia-108. Both strains carried the two signature amino acids for ‘Baltic/Siberian’ TBEV strains in the E gene (Golovljova et al., 2008). Their nucleotide identity was 96 and 98 % in the E (1223 nt) and NS3 (604 nt) genes, respectively. Notably, they clustered differently in the partial E gene-based phylogenetic tree (Fig. 1b), and these two strains, isolated from the same forest on the same day, were not related more closely to each other than to other closest relatives from distant locations; Karelia-94 was 97 % identical to strains from Yaroslavl (published in GenBank) and Karelia-108 resembled more virus strains from Vologda (GenBank) than Karelia-94. These differences between the two strains from the same forest might indicate several independent introductions of TBEV. In general, TBEV-Sib strains seem to vary more than TBEV-Eur strains, and within the Siberian subtype there is clear geographical division between Baltic and Siberian lineages (Golovljova et al., 2008). The Karelian strains described here make a branch of their own within the ‘Baltic/Siberian’ subgroup based on the partial NS3 gene analysis (Fig. 1c); the NS3 region has been suggested to give a topology that best resembles the topology of full-length genomes of flaviviruses (Billoir et al., 2000; Cook & Holmes, 2006).

Three of 296 I. persulcatus ticks from the Republic of Buryatia were positive for TBEV RNA. Previous studies have suggested that Buryatia is on a transition zone of the Siberian and Far Eastern TBEV subtypes (Pogodina et al., 2004). Together with strain 886-84 from Clethrionomys rufocanus from Irkutsk, the Buryatian TBEV strains 740-84 and 711-84 from C. rufocanus and 617-90 from I. persulcatus form a branch within the Far Eastern clade in a phylogenetic tree based on the partial E gene (Fig. 1b). We managed to isolate one TBEV-FE strain, Buryatia-169. Based on the partial E gene obtained, it was only 86–87 % identical to strains from nearby locations isolated 15–20 years ago. In addition to the isolated TBEV-FE strain Buryatia-169, we had two other TBEV RNA-positive Buryatian ticks. Based on limited sequence data available (92 nt from the 5’NCR; Table 2), the other (Buryatia-171) appeared to be a TBEV-FE strain, but quite different from the isolated Buryatia-169.

In conclusion, we studied tick panels in Finland and Russia for the presence of TBEV and isolated and characterized ten novel TBEV strains from ticks and human sera from a wide geographical area. As we used the same methodology for the different panels, the results should be comparable between the TBE-endemic regions and showed around 1 % TBEV prevalence. I. persulcatus-carried TBEV-Sib and TBEV-FE strains were isolated in the republics of Karelia and Buryatia, respectively. In Finland, in addition to I. ricinus in southern parts of the country, I. persulcatus was found on the western coast. Sequence variation, especially of the TBEV-Eur strains, was low and they rarely showed any geographical clustering, compatible with recent spread of the virus strains, possibly by migratory birds.

Acknowledgements

We thank staff from the Viral Zoonosis Research Group, Haartman Institute, University of Helsinki, Finland, and the Åke Lundkvist laboratory, Karolinska Institute, Sweden, and Anna Andreeva, Anna Petrova, Helen Dubinina, Severi Vapalahti and Hans Hästbacka for participating in the tick-sample collecting. We thank the diagnostic laboratory of the Department of Virology, HUSLAB, for providing the patient serum samples, and Dr Antti Altalo and others in the Infectious Disease Surveillance and Control team of the Finnish National Institute of Health and Welfare for contributing data. Essi Hasu, Eili Huhtamo, Tytti Manni, Pirjo Sarjakivi and Elina Tonteri are acknowledged for excellent technical support. This work has been supported financially by Helsinki University Funds, Academy of Finland, Helsinki Biomedical Graduate School, Emil Aaltonen Foundation, Finnish Cultural Foundation, Paulo Foundation, Orion-Farmos Research Foundation, Baxter Oy and the Medical Research Fund of the Åland Culture Foundation.

References


