Siberian Subtype Tickborne Encephalitis Virus, Finland

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We isolated 11 Siberian subtype tickborne encephalitis virus (TBEV) strains from Ixodes persulcatus ticks from a TBEV-endemic focus in the Kokkola Archipelago, western Finland. Thus I. persulcatus and the Siberian TBEV are reported in a focus considerably northwest of their previously known range in eastern Europe and Siberia.

Tickborne encephalitis (TBE) is a disease endemic in a zone extending from central and eastern Europe to Siberia and Japan. Three subtypes of the causative agent tickborne encephalitis virus (TBEV) are known: the European, Siberian, and Far Eastern (1,2). The main vector for the European subtype is Ixodes ricinus, and for the other 2 subtypes, I. persulcatus (1,3–5). I. ricinus is found in Europe and Middle East (6), and I. persulcatus ranges from eastern Europe to China and Japan. The boundary between their distribution lies at the Russian side of the Finnish-Russian border (1,7). The distribution areas of both tick species overlap in eastern Europe (4,5) (Figure 1). I. persulcatus has not been reported from northern or western Europe except for an engorged nymph on a willow warbler (Phylloscopus trochilus) in northeastern Sweden in May 1992 (6).

In Finland, the TBE-endemic areas are mostly in the coastal regions: two thirds of cases come from the Åland Islands. Other TBE-endemic regions include the Archipelago of Turku, a focus in Isoasari (an island outside Helsinki), the Lappeenranta region in southeastern Finland, and the Archipelago of Kokkola in western Finland (9). The virus was found in the same areas (except for Isoasari) as early as the 1960s by screening antibodies to TBEV from cattle sera (10).

The TBE focus in the Archipelago of Kokkola (63°50’N, 23°10’E), 300 km south of the Arctic Circle, has a peculiar location; it is an isolated focus, far from other TBE-endemic areas, and is the northernmost TBE-endemic area known. Furthermore, the recent TBE cases have been severe with sequelae (11,12). A cluster of cases in 2002 led us to study ticks in the Kokkola Archipelago for TBEV.

The Study

A total of 1,181 ticks were collected by flagging in the Archipelago of Kokkola in June 2004 (Table 1). In the tick-collecting areas 1–7, TBE patients had reported tick bites, and in areas 8–10, no TBE cases have been found. All the locations were islands or peninsulas within 20 km of each other.

The ticks were homogenized in pools of 10 with Dulbecco phosphate-buffered saline plus 0.2% bovine serum albumin (D-PBS-BSA) and sand to 122 pools. RNA was isolated from 100 µL of the pools by TriPure Isolation Reagent (Roche Diagnostics, Espoo, Finland). The RNA was dissolved in 20 µL diethyl pyrocarbonate–treated water, and 10 µL was used for nested reverse transcription (RT)–PCR, amplifying a 252-nt sequence from the TBEV-NS5 gene to detect TBEV-RNA according to Puchhammer-Stöckl et al. (13), except that the outer forward primer used was 5′-GGAGGCTGAACAACTG-CAC-3′. TBEV-RNA was detected in 13 pools (each consisted of 10 adult ticks) (Table 1). Assuming that only

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Figure 1. The known distribution of tickborne encephalitis (TBE)–virus endemic areas and Ixodes ticks in northern Europe. Yellow: TBE-endemic areas, adapted from International Scientific Working Group on Tick-Borne Encephalitis (8). To the south and west from the solid line, Ixodes ricinus distribution; to the east from the dashed line, I. persulcatus distribution; Lpr, Lappeenranta; EST, Estonia; LV, Latvia; LT, Lithuania.
A tick in a positive pool was positive for TBEV RNA, the overall TBEV prevalence was 1.1%.

To isolate TBEV strains from the RT-PCR–positive tick pools, 20 μL of the supernatant of the pools diluted 1:1 in D-PBS-BSA was injected intracerebrally into suckling NMRI mice. One litter of suckling mice was used for each pool. The mice were followed for 14 days or until symptoms of illness appeared, and then they were killed. From 200 μL of the homogenized mouse brains, diluted 1:5 with D-PBS-BSA, RNA was extracted by TriPure, and RT-PCR for the partial TBEV-E gene was performed. The cDNA was produced with the reverse primer 5′-CCYCCAGC-CARGAGRAAGC-3′ by M-MuLV-RT enzyme (Fermentas, Vilnius, Lithuania), and subsequent PCR was performed with this and a forward primer 5′-AACAGGGAYTTTGTCACTGGYACTC-3′ by Taq DNA polymerase (Fermentas) (detailed RT-PCR protocol available from the authors upon request).

A region of 205 nt from the NS5 gene from the RT-PCR–positive tick pools and 1,225-nt stretch from the E gene from the brains of the infected suckling mice were sequenced (GenBank accession nos. in Table 2). Unexpectedly, based on the partial NS5 sequences from the RT-PCR–positive tick pools (data not shown), the TBEV strains in Kokkola belonged to the Siberian subtype of TBEV. A phylogenetic tree based on the partial E gene sequences (1,076 nt) obtained from the TBEV isolates was prepared by the maximum likelihood method (Figure 2, scripts and datasets available from the authors upon request).

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Conclusions

A TBE focus has existed in the Kokkola Archipelago at least since the 1960s when TBEV antibodies were detected in cattle (10), but the local TBE viruses have not been characterized. A cluster of severe human cases in the beginning of the 2000s prompted us to carry out the present study. In June 2004, we collected 1,181 ticks from the Archipelago of Kokkola and detected TBEV-RNA in 13 pools. Eleven were also positive in virus isolation. The sequences showed that the strains belonged to the Siberian subtype, whereas in the other Finnish TBE-endemic foci, only European subtype TBEV closely related to the central European strains has previously been detected (15). Furthermore, the tick species was I. persulcatus. Our results show that both I. persulcatus and the Siberian type TBEV are occurring several hundreds of kilometers further to northwest than what has been known previously. Because the 2 tick species are similar to the naked eye and in their behavior, and published data on distribution of Ixodes ticks in Finland are sparse and outdated, we cannot exclude the possibility that I. persulcatus also exists unnoticed elsewhere in Finland. More tick surveys and epidemiologic studies are needed to map the distribution areas of the 2 vector species and of the different TBEV subtypes in Finland. However, in our recent tick collections from

| Location | TBE case-patient, sex/age/yr | No. ticks (n/l/m) | No. tick pools | Tick pools positive in RT-PCR† | Tick pools positive in virus isolation† |
|----------|-------------------------------|------------------|----------------|-------------------------------|---------------------------------------|
| 1        | M/48/2004 (12)                | 184 (19/87/76)   | 19             | 4, 8, 9                       | 8, 9                                  |
| 2        | M/21/2002 (11)                | 80 (24/25/31)    | 8              | 25, 26                        | 25, 26                                |
| 3        | F/24/2002 (11)                | 158 (2/87/69)    | 16             | 39                            | 39                                    |
| 4        | F/12/2002 (11)                | 474 (16/227/231) | 48             | 79, 81, 84, 86, 86, 102, 118   | 79, 81, 84, 86, 102, 118              |
| 5        | M/7/2003                      | 166 (6/83/77)    | 17             | –                             | ND                                    |
| 6        | M/7/2003                      | 6 (0/3/3)        | 1              | –                             | ND                                    |
| 7        | No known cases                | 55 (1/25/29)     | 6              | –                             | ND                                    |
| 8        | No known cases                | 9 (2/5/2)        | 1              | –                             | ND                                    |
| 10       | No known cases                | 8 (0/6/2)        | 1              | –                             | ND                                    |

*TBE, tickborne encephalitis; RT-PCR, reverse transcription–polymerase chain reaction; n, nymphs; f, adult females; m, adult males; ND, not determined.
†Nos. indicate the tick pool numbers, the same as used in the phylogenetic tree.
Åland and southern (Isosaari, 60°N, 25°E) and eastern (Lappeenranta, 61°N, 28°E, and Joensuu, 62°N, 29°E) Finland, all ticks were *I. ricinus*.

Some researchers have found indications that the Siberian TBEV might cause more severe or more persistent forms of TBE than the European subtype (4), and 3 of 5 recent human TBE cases in Kokkola have been severe (11,12). However, the number of cases studied from Kokkola is too small for firm conclusions on the severity of the local disease.

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### Table 2. TBE virus strains compared by sequence analysis*

| Strain   | Geographic origin | GenBank accession no. |
|----------|-------------------|-----------------------|
| Kokkola 4| Location 1, Kokkola | DQ451297†            |
| Kokkola 8| Location 1, Kokkola | DQ451296†            |
|          |                   | DQ451298‡            |
| Kokkola 9| Location 1, Kokkola | DQ451299†            |
| Kokkola 25| Location 2, Kokkola| DQ451300†            |
| Kokkola 26| Location 2, Kokkola| DQ451289†            |
| Kokkola 39| Location 3, Kokkola| DQ451301†            |
| Kokkola 79| Location 4, Kokkola| DQ451302†            |
| Kokkola 81| Location 4, Kokkola| DQ451303†            |
| Kokkola 84| Location 4, Kokkola| DQ451291†            |
| Kokkola 85| Location 4, Kokkola| DQ451304†            |
| Kokkola 86| Location 4, Kokkola| DQ451292‡            |
| Kokkola 102| Location 4, Kokkola| DQ451293‡            |
| Kokkola 118| Location 4, Kokkola| DQ451295‡            |

| Iso40  | Isosaari, Finland | AJ298323              |
| Kumlinge A 52 | Aland, Finland | X80286                |
| Est54 | Estonia           | DQ393773              |
| Est3535 | Estonia         | DQ393774              |
| Est2546 | Estonia         | DQ393779              |
| Est3476 | Estonia         | DQ393776              |
| Latvia 1–96 | Latvia        | AJ415565              |
| RK1424 | Latvia            | AF091016              |
| Neudoerfl | Austria        | U27495                |
| Hypr   | Czech Republic   | U39292                |
| 263    | Czech Republic   | U27491                |
| Zausaev| Siberia, Russia  | AF527415              |
| Laschenko | Novosibirsk, Russia | L40361              |
| Aina   | Irkutsk, Russia  | AF091006              |
| EK-328 | Estonia          | DQ486861              |
| TBEV228| Novosibirsk region, Russia | DQ385498 |
| TBEV1467 | Novosibirsk region, Russia | AY753582 |
| Sofin-HO | Primorskiy Kray, Russia | AB062064 |
| Oshima 5–10 | Hokkaido, Japan | AB062063              |
| LIV    | United Kingdom   | NC 001809             |
| OHFV, strain | Russia          | AY193805              |
| Langat | Malaysia          | AF253419              |
| Powassan, LB strain | United States | NC 003687              |

*†Accession no. for the partial NS5 gene sequence.
‡Accession no. for the partial E gene sequence.
§Accession no. for the partial NS5 gene sequence.

**Figure 2. Maximum likelihood phylogenetic tree of partial E gene (1,076 nt). The bar below indicates the nucleotide substitutions per site. The accession nos. of the strains used can be seen in Table 2. The bootstrap support values <50 are not shown.**
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