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European Subtype
Tick-borne Encephalitis Virus
in Ixodes persulcatus Ticks

To the Editor: The northernmost tick-borne encephalitis (TBE) focus is in Simo, Finnish Lapland. Four TBE cases were confirmed during 2008–2009. Tick-borne encephalitis virus (TBEV) is transmitted by Ixodes spp. ticks and is endemic to Eurasia from central Europe to the Far East. The virus has 3 subtypes: European (TBE-V-Eur), Siberian (TBE-Sib), and Far Eastern (TBE-FE). TBEV-Eur is mainly transmitted by I. ricinus ticks (sheep ticks) and the 2 other subtypes by I. persulcatus ticks (taiga ticks). The range of I. ricinus ticks covers most of continental Europe and the British Isles; I. persulcatus ticks are distributed throughout eastern Europe and Asia to the People’s Republic of China and Japan.

The transmission cycle of at least TBEV-Eur in nature is fragile and depends on microclimatic conditions. Thus, within the I. ricinus distribution area, TBE is endemic merely focally (1,2). In Finland, TBE foci are located by the sea or large lakes (online Appendix Figure, www.cdc.gov/EID/content/17/2/321-appF.htm). Both vector tick species are found: I. ricinus ticks in scattered foci along the western coast, including the Kokkola archipelago and Närpiö municipality, where they carry TBEV-Sib (3,4) (online Appendix Figure).

The first human TBE cases from Simo in Lapland (65°40′N, 24°54′E; online Appendix Figure) were reported during 2008 (n = 2) and 2009 (n = 2). On the basis of interviews with the 2 patients in 2008, we collected 97 ticks and 17 bank voles from the 2 probable sites of infection during June 2009. From the rodents, we extracted

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blood from the heart and performed TBEV-antibody tests by immunofluorescence assay. The ticks were placed in 51 pools (1–3 ticks/pool). We isolated RNA from tick pools and rodent lungs and brains by TriPure Isolation Reagent (Roche Diagnostics, Indianapolis, IN, USA) and performed real-time reverse transcription–PCR (5) to detect TBEV RNA. For the positive tick pools, we confirmed the identification species by *Ixodes* mtDNA sequencing (6).

Six of 51 tick pools (with a total of 97 *I. persulcatus* ticks) were positive for TBEV in real-time reverse transcription–PCR, resulting in 6% TBEV RNA prevalence. At least 1 organ was positive for TBEV RNA in as many as 15/17 bank voles, in line with our finding that TBEV RNA persists in rodents for months (7); 4 rodents had antibodies to TBEV. The TBEV RNA prevalence among ticks and rodents was relatively high, as is the incidence among humans (0.57 cases/year/1,000 inhabitants) in Simo, indicating a focus with high activity.

We isolated 6 TBEV strains from suckling mice (experimental animal permit ESLH-2008–06558/ Ym-23): 2 from *I. persulcatus* tick pools (Simo-38 and Simo-48; pools of 2 and 3 ticks, respectively), and 4 from TBEV antibody– and RNA–positive rodent lung–brain suspensions (Simo-2, -5, -7 and -9). Partial envelope (E) and nonstructural protein 3 gene sequences were identified, belonging to the TBEV-Eur subtype. The partial nonstructural protein 3 gene sequences were identical, and the phylogenetic tree showed similar topography as for the E gene (not shown).

The only tick species found in Simo was *I. persulcatus*, further widening its known distribution along the western coast of Finland (online Appendix Figure). However, the virus subtype found in Simo was TBEV-Eur strain, the main vector of which is the *I. ricinus* tick.

TBEV-Eur strains are commonly very closely related to each other and do not form clear geographic clusters (4). Thus, it is difficult to deduce the origin of the virus. The nearest TBE-endemic focus is the Kokkola archipelago, ≈200 km south (online Appendix Figure), but there *I. persulcatus* ticks carry the TBEV-Sib strain (3). The nearest areas to which the TBEV-Eur strain is endemic are in southern Finland where only *I. ricinus* ticks have been found.

Cattle serum samples were negative for antibodies to TBEV in the Simo area in the 1960s (8). The first human TBE cases from Simo were identified during 2008 and 2009. We isolated TBEV strains from ticks and rodents in 2009. Simo appears to be a recently established, and the northernmost, TBE focus known. TBEV may have been introduced to Simo from a geographically distinct location recently, likely within the past 50 years.

TBE seems to be moving northward in Europe (9) and shifting upward to higher elevations in the mountains (10), apparently influenced by climate change. An altered microclimate favoring TBE circulation (1), in addition to introduction of the virus, could also explain the recent emergence of TBE in Simo. In conclusion, Simo in Finnish Lapland is a new TBE-endemic focus demonstrating northward movement of foci and an unusual combination of the TBEV-Eur strain and *I. persulcatus* ticks in an area with no evidence of cocirculation of tick species or TBEV subtypes.

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Rickettsia aeschlimannii in Hyalomma marginatum Ticks, Germany

To the Editor: Rickettsia spp. of the spotted fever group cause worldwide emerging human infections known as tick-borne rickettsioses (1). Data on the occurrence and prevalence of Rickettsia in Germany are still limited (2). Six Rickettsia species have been reported to date (2). R. helvetica, R. felis, R. massiliae, and R. monacensis were detected with a relatively low prevalence in Ixodes ricinus ticks collected in southern Germany (2). R. raoultii was identified with high prevalence in the rapidly expanding area where D. reticulatus ticks are found (2). R. raoultii was recently recognized as an agent of tick-borne lymphadenopathy/Dermacentor-borne necrosis and erythema lymphadenopathy (3). Low prevalence of another tick-borne lymphadenopathy agent, R. slovaca, in Dermacentor marginatus ticks collected in southern Germany was recently reported (4).

We report the detection in Germany of the pathogenic SFG species R. aeschlimannii (1), which is phylogenetically close to R. raoultii and causes an infection with clinical signs similar to those of Mediterranean spotted fever (1). To determine the prevalence of R. raoultii in the ticks in Berlin/Brandenburg and neighboring regions, we collected 294 ticks; 288 had been collected either from vegetation or domestic animals and morphologically identified as adult D. reticulatus ticks. The remaining 6 ticks were delivered by an ornithologist who had removed them from a bird (belonging to the genus Acrocephalus spp.) that he had captured in the reeds near Pakendorf and Zerbst, Saxony-Anhalt, in May 2007. These 6 ticks were reported as D. reticulatus–like adults but were damaged in the collection process, making an exact morphologic evaluation impossible.

DNA was isolated from the complete tick body by homogenization in the SpeedMill (Analytik Jena Biosolutions, Jena, Germany) followed by purification by RapideStripe tick DNA/RNA Extraction Kit (Analytik Jena Biosolutions). Multispacer typing (5) was used for molecular detection and determination of Rickettsia spp. (Figure). DNA sequencing and analysis were performed as described (Figure).

In 51.3% of the intact tick isolates, R. raoultii was detected. In each of the 3 damaged isolates, the multispacer type pattern was detected, which was 100% identical to that of R. aeschlimannii (5) (Figure). Moreover, PCR, which we routinely use as a positive control for molecular identification of D. reticulatus, yielded no product in the damaged isolates (Figure).

To determine the species of the damaged ticks, we used 3 tick-specific PCRs. One amplified a 16S rRNA fragment used for phylogenetic studies of ticks (6). Use of the other 2 PCRs was