diagnosed cases ranged from July 20 to October 4, 2009. Cases peaked in early September and subsided in early October. The median age of patients with confirmed or probable disease was 47.3 years (range 3–96 years). Infections occurred in all age groups, but most infections occurred among persons 41 to 65 years of age; 118 (60.2%) were women, and 172 were farm workers.

Confirmed and clinically diagnosed cases occurred in 18 villages, which were part of 7 towns. Most cases (182) were reported in Yiting, where the first case was confirmed, and in particular, were in persons who lived in the villages of Qingsu, 100 cases; Fantianzhu, 49 cases; Xitian, 19 cases; Shangzhai, 4 cases; and Xiateng, 4 cases.

The outbreak shows that DENV-3 subtype III is easily transmitted among humans and mosquitoes and can adapt efficiently to a new area. Other countries where the climate is similar to that of Zhejiang Province (subtropical monsoon) should be aware of the risk for expansion of DENV-3 subtype III transmission. Clinical vigilance and strong epidemiologic and laboratory surveillance are essential**.**

Acknowledgments

We thank the physicians and staff at Health Bureau of Zhejiang Province, the Yiwu Community Health Centers, the Yiwu Department of Health Service, and Yiwu Center for Disease Control and Prevention for their support and assistance with this investigation.

Jimin Sun,[1] Junfen Lin,[1] Juying Yan, Weizhong Fan, Liang Lu, Huakun Lv, Juan Hou, Feng Ling, Tao Fu, Zhiping Chen, Liming Cong, Qiyong Liu, Yanjun Zhang, and Chengliang Chai

[1]These authors contributed equally to this article.

Author affiliations: Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou, People's Republic of China (J. Sun, J. Lin, J. Yan, J. Hou, F. Ling, Z. Chen, L. Cong, Y. Zhang, C. Chai); Yiwu Municipal Center for Disease Control and Prevention , Yiwu, People's Republic of China (W. Fan, T. Fu); and State Key Laboratory for Infectious Disease Prevention and Control, Beijing, People's Republic of China (Q. Liu)

DOI: 10.3201/eid1702.100396

References

1. Gubler DJ, Sather GE, Kuno G, Cabral JR. Dengue 3 virus transmission in Africa. Am J Trop Med Hyg. 1986;35:1280–4.
2. Kanesa-thasan N, Chang GJ, Smoak BL, Magill A, Burrous MJ, Hoke CHJ. Molecular and epidemiologic analysis of dengue virus isolates from Somalia. Emerg Infect Dis. 1998;4:299–303. DOI: 10.3201/eid0402.980220
3. Qiu FX, Gubler DJ, Liu JC, Chen QQ. Dengue in China: a clinical review. Bull World Health Organ. 1993;71:349–59.
4. Juying Y, Yiyu L, Jingqing W, Haiyan M, Yan F, Wen SH, et al. The etiological study of a dengue fever outbreak and the molecular characterization of the dengue virus isolates in Zhejiang Province. Chin J Virol. 2006;22:339–44.
5. Lei L. Comparison of epidemiological characteristics of dengue fever between 2002 and 2006, Guangzhou. S China J Prev Med. 2008;34:18–21.
6. Shaojian C, Rongtao H, Nengxiong Z, Jianming O, Yansheng Y. Epidemiological analysis of dengue fever outbreak in 2004 from Fujian Province. Haixia J Prev Med. 2006;12:32–4.
7. Swofford DL. PAUP*: phylogenetic analysis using parsimony and other methods, Version 4.0b9. Sunderland (MA): Sinauer Associates; 2002.

Address for correspondence: Jimin Sun, Zhejiang Centers for Disease Control, Xinching Rd, Binjiang District, Hangzhou City, People's Republic of China 310051; email: sunjimin1981@yahoo.com.cn

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 (TBEV-Eur), Siberian (TBEV-Sib), and Far Eastern (TBEV-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 the southern and central parts of the country, but *I. persulcatus* ticks are 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 from 2008, we collected 97 ticks and 17 bank voles from the 2 probable sites of infection during June 2009. From the rodents, we extracted

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 genes (*4*) of the isolated TBEV strains were sequenced (accession nos. HQ228014–HQ228024, GenBank) and subjected to phylogenetic analysis (online Appendix Figure). Within the 1208 nt from the E gene, Simo-38 and Simo-48 from ticks and Simo-9 from a bank vole were identical. Other sequences differed for 1 nt and Simo-2 for 1 aa compared with the others. All strains were monophyletic and belonged 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.

Acknowledgments

We thank Agnė Alminaitė, Miska Merentie, Maria Razzauti Sanfeliu, and Liina Voutilainen for participating in tick and rodent collection, and Eili Huhtamo and Paula Kinnunen for excellent technical assistance.

Baxter Oy, Outokumpu Stainless Oy, and the Finnish Cultural Foundation are acknowledged for financial support.

Anu E. Jääskeläinen, Elina Tonteri, Tarja Sironen, Laura Pakarinen, Antti Vaheri, and Olli Vapalahti

Author affiliations: University of Helsinki, Helsinki, Finland (A.E. Jääskeläinen, E. Tonteri, T. Sironen, A. Vaheri, O. Vapalahti) National Institute for Health and Welfare, Helsinki (L. Pakarinen); and Hospital District of Helsinki and Uusimaa, Helsinki (A. Vaheri, O. Vapalahti)

DOI: 10.3201/eid1702.101487

References

1. Randolph SE, Green RM, Peacey MF, Rogers DJ. Seasonal synchrony: the key to tick-borne encephalitis foci identified by satellite data. Parasitology. 2000;121:15–23. DOI: 10.1017/S0031182099006083
2. Lindquist L, Vapalahti O. Tick-borne encephalitis. Lancet. 2008;371:1861–71. DOI: 10.1016/S0140-6736(08)60800-4
3. Jääskeläinen AE, Tikkakoski T, Uzcátegui NY, Alekseev AN, Vaheri A, Vapalahti O. Siberian subtype tickborne encephalitis virus, Finland. Emerg Infect Dis. 2006;12:1568–71.
4. Jääskeläinen AE, Sironen T, Murueva GB, Subbotina N, Alekseev AN, Castrén J, et al. Tick-borne encephalitis virus in ticks in Finland, Russian Karelia, and Buryatia. J Gen Virol. 2010;91:2706–12. DOI: 10.1099/vir.0.023663-0
5. Schwaiger M, Cassinotti P. Development of a quantitative real-time RT-PCR assay with internal control for the laboratory detection of tick borne encephalitis virus (TBEV) RNA. J Clin Virol. 2003;27:136–45. DOI: 10.1016/S1386-6532(02)00168-3
6. Caporale DA, Rich SM, Spielman A, Telford SR III, Kocher TD. Discriminating between *Ixodes* ticks by means of mitochondrial DNA sequences. Mol Phylogenet Evol. 1995;4:361–5. DOI: 10.1006/mpev.1995.1033

7. Tonteri E, Jääskeläinen AE, Tikkakoski T, Voutilainen L, Niemimaa J, Henttonen H, et al. Tick-borne encephalitis virus in wild rodents in winter, Finland, 2008–2009. Emerg Infect Dis. 2011;17:72–75.
8. Tuomi J, Brummer-Korvenkontio M. Antibodies against viruses of the tick-borne encephalitis group in cattle sera in Finland. Ann Med Exp Biol Fenn. 1965;43:149–54.
9. Randolph SE, Rogers DJ. Fragile transmission cycles of tick-borne encephalitis virus may be disrupted by predicted climate change. Proc Biol Sci. 2000;267:1741–4. DOI: 10.1098/rspb.2000.1204
10. Lukan M, Bullova E, Petko B. Climate warming and tick-borne encephalitis, Slovakia. Emerg Infect Dis. 2010;16:524–6. DOI: 10.3201/eid1603.081364

Address for correspondence: Anu E. Jääskeläinen, Department of Virology, Haartman Institute, PO Box 21 FI-00014, University of Helsinki, Helsinki 00014, Finland; email: anu.jaaskelainen@helsinki.fi

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. aeshlimannii* (*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 *Acrocephalus scirpaceus* 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

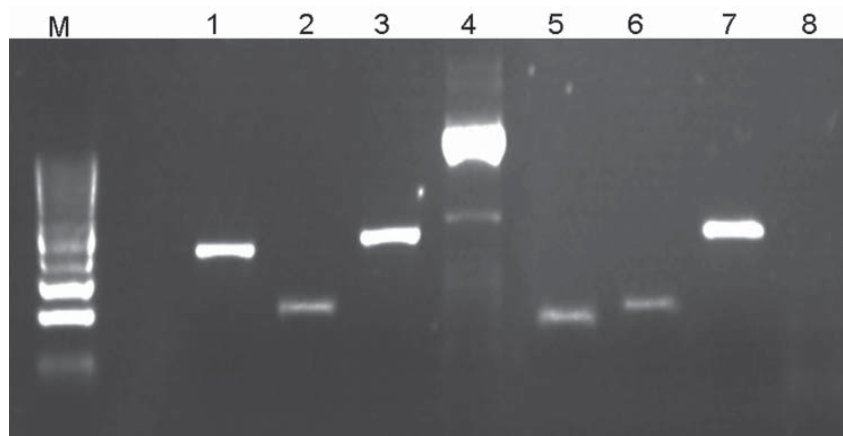

Figure. Illustration of multispacer typing. Amplicons 1–4 result from PCRs on DNA obtained from 1 *Rickettsia raoultii*–infected *Dermacentor reticulatus* tick isolate; lanes 5–8 result from PCRs on 1 damaged isolate. PCRs amplifying dksA-xerC (lanes 1 and 5), mppA-purC (lanes 2 and 6), and rpmE-tRNA (lanes 3 and 7) intergenic spacers were performed as described (*5*). PCR amplifying the entire internal transcribed factor 2 (ITS2) locus of *D. reticulatus* tick (lanes 4 and 8) was involved in each PCR run as a positive control and for validation of *D. reticulatus* tick identity (the primers will be described elsewhere).The negative result of ITS2 PCR with the damaged isolates (lane 8) indicated that they are not *D. reticulatus* ticks. Lane M, DNA size marker (100-bp ladder). PCR products were directly sequenced in both directions with respective primers by an ABI PRISM DNA Sequencer (Applied Biosystems, Foster City, CA, USA). DNA Star package (DNA Star, Madison, WI, USA) and the tools offered by the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) were used for DNA search and analysis.