

increased during the past 13 years (6). This trend is accompanied by an increase in the total number of periprosthetic joint infections, even though the overall percentage of this complication is low (7). The most commonly isolated organisms in periprosthetic joint infections are gram-positive cocci, specifically *Staphylococcus aureus* and *S. epidermidis* (8). In a retrospective review, Moran et al. (9) examined the microbiological spectrum of 112 patients undergoing debridement and irrigation for a periprosthetic joint infection (hip [52], knee [51], elbow [4], ankle [3], shoulder [2]) at a tertiary care center in the United Kingdom during 1998–2003. The most frequently isolated microorganisms were coagulase-negative staphylococci (47%) followed by methicillin-sensitive *S. aureus* (44%), methicillin-resistant *S. aureus* (8%), aerobic gram-negative organisms (8%), and anaerobes (7%). Thirty-seven percent of patient specimens grew multiple microorganisms.

We document the ability of *B. extructa* to cause an infection beyond its usual habitat, the oral flora. We hypothesize that the infection in this patient might have developed from hematogenous seeding in which an undiscovered and asymptomatic oral infectious nidus might have served as the seeding focus while mild trauma to the hip could have facilitated access to the joint space.

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## Tick-borne Encephalitis Virus, Zealand, Denmark, 2011

**To the Editor:** In Scandinavia, the incidence of tick-borne encephalitis (TBE) is increasing and expanding its geographic range (1). TBE virus (TBEV) types TBEV-Eur and TBEV-Sib occur in Estonia and Finland, along with 2 tick species, *Ixodes persulcatus* and *I. ricinus*. In Denmark, TBE has been reported since the 1950s only from the isolated Bornholm Island in the Baltic Sea with an incidence of ≈4 cases per 100,000 persons (2). Statistical climate-matching models based on the known spatial distribution of TBEV indicate that the present North Zealand climate also would support TBEV-Eur transmission cycles (3). Recently (2008 and 2009), we reported TBE in 2 persons who had histories of tick-bite and originated from a single location in a small forest area (Tokkekøb Hegn) in North Zealand where TBE was previously unrecognized (4).

To determine whether TBE was established in this possibly new TBE focus in mainland Denmark outside Bornholm, we collected ticks by flagging (4) from 3 sites at Tokkekøb during June–July 2011. The 3 sites yielded 896 ticks (854 nymphs, 22 male adults, 20 female adults) in 24 pools. A fourth site at Grib Forest 10 km to the north yielded 198 ticks (183 nymphs, 9 male adult, 6 female adults) in 13 pools.

Flagging was repeated in September 2011 at Tokkekøb to confirm the presence of TBEV and to obtain material suitable for virus isolation. Here, we obtained 7 pools (100 nymphs each) and 1 pool with adults (15 male, 15 female). In September 2011, we also obtained 13 pools (738 nymphs, 37 male adults, 41 female adults) at 3 suspected TBE locations on Bornholm Island. In addition, 1,073 ticks in 58 pools were collected in 2010 and 2011 from deer inspected by the National Center for Wildlife Health from 54 various locations (Figure, panel A). All ticks were identified as *I. ricinus* on the basis of morphology. For TBEV-specific real-time PCR (5), ticks were homogenized in 0.5 mL nucleic acid extraction buffer and RNA/DNA extracted from 0.2 mL homogenate by using the MagNA Pure total NA kit (Roche, Indianapolis, IN, USA). Three of 37 pools (2 with nymphs, 1 with adult females) from Tokkekøb were TBEV RNA positive. None of 58 tick pools from other locations in Denmark or Bornholm were positive for TBEV but contained other pathogens (6). Five of the 8 pools obtained from the second

flagging session (all nymphs) in Tokkekøb were TBEV PCR positive, and 2 yielded isolates (T2, T3) in VeroB4 cell culture. Considering that the duration of the nymphal stage in *I. ricinus* is usually only 1 or 2 years in northern Europe (7), the repeated identification of TBEV in nymphs at the same location in 2009 and 2011 indicates establishment of a new focus of endemic TBEV in Denmark.

Phylogenetic analysis of TBEV-E sequences (1,488 nt) of central European (8) and Scandinavian TBEV strains did not group the Zealand isolate T2 (T3 was not sequenced) with the Bornholm strain but into a subclade with 2 isolates from Sweden, Torö-2003 (9) (GenBank accession no. DQ401140) and Saringe-2009 (GenBank accession no. KC469073); an isolate from Norway (GenBank accession no. EF565947), and isolates from North Bohemia (Czech Republic). The Bornholm strain located into a different subclade containing various sequences from South and Central Bohemia (Figure, panel B). TBEV sequences from the Baltics and Finland

locate to a spate clade. The missing link between the isolates from Bornholm and Zealand also was observed in a median joining network analysis (Splits Tree program, Epsilon1 [www.splitstree.org], 2,000 iterations [data not shown]).

Two severe clinical cases of TBE connected to this new focus occurred in 2008–2009 (4). To search for additional missed clinical TBE cases from this area, we examined serum and cerebrospinal fluid of 96 patients (2007–2009) in whom encephalitis developed after tick bite; these samples were found negative for *Borrelia* spp. by antibody ELISA and PCR (online Technical Appendix, wwwnc.cdc.gov/EID/article/19/7/13-0092-Techapp1.pdf). To assess anti-TBEV seroprevalence, we also tested serum from 78 patients experiencing “summer flu” who had histories of tick bite; this serum was submitted by general practitioners in North Zealand during July–November 2010 (online Technical Appendix). Except for 1 patient infected in Bornholm and 2 patients infected in Sweden, none were

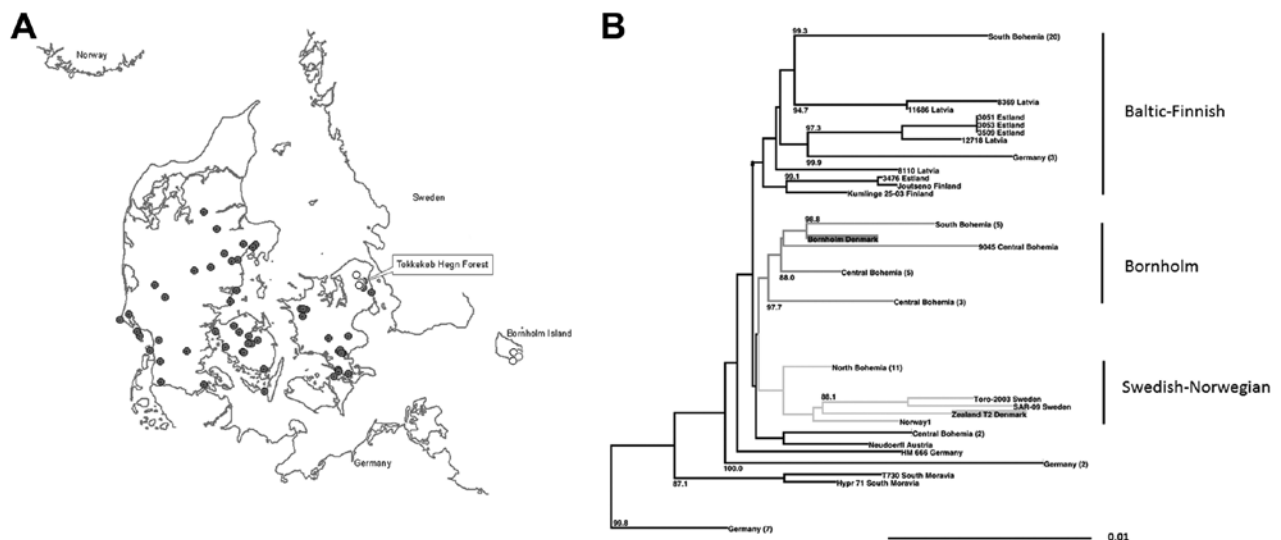


Figure. A) Tick collection areas in Denmark. Red indicates ticks sampled from animals; blue indicates flagging. B) Neighbor-joining phylogenetic analysis of a 1,488-nt set of 78 tick-borne encephalitis virus (TBEV)–Eur E gene sequences including reference strains Neudoerfl (Austria) and Hypr 71 (South Moravia) performed in ClustalW with a 1,000 bootstrap approach (LASERGENE, MEGALIGN, DENDROSCOPE) outgrouped to Louping ill virus (data not shown). Sequence designations of central European strains as in (8). Dark gray indicates Denmark Bornholm clade; white indicates Swedish-Norwegian clade. For simplicity, some subtrees were collapsed; these are designated with region and number of sequences in the collapsed subtree in brackets.

positive by ELISA (Enzygnost Anti-TBE/FSME Virus [IgG, IgM] Siemens, Erlangen, Germany) or PCR (online Technical Appendix). Since the 1980s, Sweden has experienced a 4-fold increase in human TBE incidence, with spread southwest (10). The emergence of the TBEV strain T2 closely related to isolates from Sweden may be a continuation of this geographic trend. A previous antibody study found 3 deer positive for TBEV in Zealand-Falster (2); however, without convincing neutralization data, this finding is not confirmed. The lack of TBEV viremia and seropositivity among the patients in Zealand who had histories of tick bites supports a recent introduction to the new focus. Thus, 2 distinct introductions of TBEV have occurred in Denmark. The underlining environmental or climatic factors driving this geographic trend remain unknown.

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## Usutu Virus in Migratory Song Thrushes, Spain

**To the Editor:** Usutu virus (USUV), a member of the Japanese encephalitis virus antigenic group, was first detected in 1959 in mosquitoes in South Africa (1), and it emerged in 1996 in blackbirds (*Turdus merula*) in Italy (2). Recent cases of USUV infection in asymptomatic blood donors (3) and severe disease in immunocompromised persons (4) have shown its zoonotic potential.

Epidemiology and molecular phylogeny of USUV isolated in Italy, Austria, Hungary, Switzerland, and Germany suggest that stable endemic mosquito–bird cycles have been established in Europe (5,6). Where active vector surveillance programs exist, USUV is detected in mosquitoes before bird deaths and human infections. USUV strains similar to African strains were detected in mosquitoes in Spain in 2006 and 2009 (7,8).

In November 2012, two live song thrushes (*Turdus philomelos*) with central nervous system signs were recovered from a die-off of ≈10 birds at a hunting estate in southern Spain. A full necropsy was conducted on the 2 thrushes (which died shortly after capture), and samples were collected for virus detection and histopathologic examination. Total RNA was extracted from oral and cloacal swab specimens, from serum from a cardiac blood clot, and from heart, kidney, spleen, and brain tissues by using High Pure RNA Tissue Kit (Roche Diagnostics, Barcelona, Spain) and analyzed by generic flavivirus SYBR Green (QIAGEN, Madrid, Spain) real-time reverse transcription PCR (RT-PCR) and by a generic conventional nested flavivirus RT-PCR (9). The product of the first PCR (1,048 bp) was resin purified, cloned into pGEM-T (Promega, WI, USA), and sequenced.

# Tick-borne Encephalitis Virus, Zealand, Denmark, 2011

## Technical Appendix

Technical Appendix Table. Overview of serum samples collected and tested for tick-borne encephalitis virus and antibodies in Denmark outside Bornholm, 2011

Variable	<i>Borrelia</i> samples	Summer flu samples
No. samples	96	79
No. patients	96	78
Sex	55 F, 41 M	46 M, 32 F
Age, y (mean)	3–87 (41)	4–84 (49)
Period	2007 Jul 3–2009 Aug 14	2010 Jul 21–2010 Nov 4
Results	2 IgG positive and 1 IgG and IgM positive	None positive in PCR or serology