First identification of tick-borne encephalitis in Denmark outside of Bornholm, August 2009

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The incidence of tick-borne encephalitis (TBE) in Scandinavia is increasing and spreading geographically. Following two clinical cases of TBE hospitalised after tick bites in northern Zealand, Denmark, specific IgM and IgG antibodies against tick-borne encephalitis virus (TBEV) were demonstrated in acute serum samples of these patients. TBEV was identified by RT-PCR in ticks collected from the same location. This is the first report of TBEV in Ixodes ricinus leading to clinical cases in Denmark outside of Bornholm island.

Background

Tick-borne encephalitis (TBE) is caused by TBE virus (TBEV), a member of genus *flavivirus*, family *Flaviviridae*. The incidence of TBE has been increasing in neighbouring countries of Denmark (Sweden and Germany) over the past years and mirrors the geographic spread and increased number of ticks [1,2]. The vector for the European subtype, TBEV-Eu, is *Ixodes ricinus* (the Common Tick) that is seen in most of Europe and is the dominant tick species in Denmark (>90%). In Denmark, TBE is endemic only on the island Bornholm in the Baltic sea, with a stable annual incidence of 4 per 100,000 inhabitants [3]. Three serum samples from roe deer from Zealand examined during the 2002-2003 hunting season were found to be antibody-positive for the TBE-complex of viruses [4]. However, a cross-reaction with Louping ill virus could not be excluded. Importantly, no clinical cases of TBE have been reported or TBEV detected outside Bornholm.

Clinical case and virological analysis

In July 2009, a man in his 40s developed fever and other influenza-like symptoms as well as arthritis about one week after receiving four tick bites in his own garden. The patient is a forest worker living in a house in the forest. After about four days of recovery he was hospitalised two weeks after the bites with symptoms of meningoencephalitis and mononuclear cells in the spinal fluid. Serum samples from the time of admission to hospital at the beginning of the encephalitis were negative for *Borrelia* but had positive IgM (optical density (OD450nm) 1,190, cut-off 224) and IgG (OD450nm 695, cut-off 224) titres to TBEV as measured by a validated ELISA (Enzygnost, Siemens) [5]. Spinal fluid was negative in the PCR for herpes simplex virus, enterovirus, varicella zoster virus and TBEV. At the time of publication of this report, the patient was recovering but continued to feel dizzy.

The patient reported about a man in his 30s who was working in a kindergarten in the same forest about 500 meters away, who had a similar unidentified viral meningoencephalitis after tick bites the year before (October 2008). When re-examining this second patients' acute serum from 2008, the antibody test was positive for anti-TBEV IgM (OD 609, cut-off 224) and IgG (OD 1,109, cut-off 243), and a recent follow-up convalescent serum (taken approximately one year later) was still IgG-positive (OD 942, cut-off 243) but IgM-negative for TBEV [5]. He was therefore rediagnosed as a TBE patient.

A TBEV antibody plaque neutralisation test (kindly performed by Dr. Matthias Niedrig, Robert Koch-Institute, Berlin) using TBEV K23 according to Reinhardt *et al*. [6] was positive on the convalescent serum but not on the acute serum drawn during the encephalitis from both patients.

Environmental analysis

Ticks were collected by "flagging"(dragging of a 1x1 m cloth through the grass) at the edge of the forest surrounding the forest worker's garden, identified by species and sorted into three pools of approximately 50 nymphs, 30 adult females and 25 adult males, respectively. Ticks were also collected (nine pools containing a total of 219 larvae and 62 nymphs and adults) at three different sites in an adjacent forest with the highest density of deer in Denmark. RNA was extracted from the ticks using MagNA Pure total NA kit (ABI), and a real-time RT-PCR was run in a quality-controlled routine PCR diagnostic laboratory using specific primers and probes as described in [7]. The PCR is specific for viruses of the TBEV complex as validated by the European Network for Diagnostic of Imported Viral Diseases, ENIVD (www.enivd.de). Only the pool of nymphs from the patient's garden was strongly positive (RT-PCR cycle threshold value of 22).

Discussion

These are the first two cases of TBE in Denmark outside Bornholm that are confirmed by identification of viruses of the TBEV complex in *I. ricinus* nymphs collected at the same location and same time of transmission to a patient. Both cases had a typical biphasic disease starting with influenza-like symptoms, easily misdiagnosed during the present influenza A(H1N1)v pandemic, and with some neurological sequelae (dizziness, fatigue) after the meningoencephalitis. Both patients were TBE IgM- and IgG-positive in the acute serum. For the patient from 2008, we had the

opportunity to obtain convalescent serum approximately one year later, which, as expected, was TBE IgG-positive and IgM-negative, and positive in the neutralisation test confirming the qualitative ELISA test. It takes time for neutralising antibodies to develop and they are normally not present during the acute illness [5].

It was expected that the distribution of TBE would expand in Europe [1,2,8], and spread in Denmark has been suggested based on serology in roe deer [4]. However, the investigation of roe deer serum antibodies has in itself limited relevance to human medicine, partly because of the uncertainty of the serology method used. So far the distribution on Zealand and the rest of Denmark is not known and could be either very local or very wide. The finding of two confirmed human cases in 2008 and 2009, respectively, suggests that TBEV has been present but unnoticed for a longer time.

According to the Danish legislation, TBE is not a notifiable disease. However, diagnostic tests for TBEV are only performed at the Department of Virology at Statens Serum Institut, and we have not seen any cases of TBE in Denmark outside Bornholm before these cases. We have begun a systematic collection of ticks in Denmark and have so far identified TBEV only in one of two likely locations in north Zealand. The PCR is specific for the TBEV complex, but in addition, we are in the process of culturing or otherwise amplifying the viruses isolated from the collected ticks in collaboration with ENIVD in order to obtain sequences for confirmation and molecular epidemiology. Further sampling, molecular characterisation of the Zealand TBEV, increased clinical awareness and continued monitoring should confirm and clarify the spread of TBE in Denmark.

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