J. Tomažič, M. Poljak, P. Popovič, M. Matičič, B. Beovič, T. Avšič-Županc, S. Lotrič, M. Jereb, F. Pikelj, N. Gale

Tick-Borne Encephalitis: Possibly a Fatal Disease in its Acute Stage. PCR Amplification of TBE RNA from Postmortem Brain Tissue

Summary: Tick-borne encephalitis has occurred regularly in Europe since it was first diagnosed in 1931 by *Schneider*. The mortality rate of patients with this disease is 1–2%. Death usually occurs in the acute stage of illness. A case report of a 28-year-old patient from Slovenia, who died shortly after the onset of tick-borne encephalitis, is described. The clinical course of disease, results of serological tests, neuropathological findings and polymerase chain reaction amplification of parts of viral genome from postmortem brain tissues are presented.

Introduction

Tick-borne encephalitis (TBE) is one of the most important human infections of the central nervous system (CNS) in Central Europe. In Central Europe and Scandinavia, TBE virus (*Flaviviridae* family) is mainly transmitted by the vector *Ixodes ricinus* [1, 2]. Typically, the disease takes a biphasic course (in approximately 75% of the cases). After the first phase (viremia), which is characterised by flu-like symptoms and followed by an asymptomatic interval, the second phase occurs in the form of CNS disease and presents as meningitis, meningoencephalitis, meningoencephalomyelitis or meningoencephaloradiculitis [3]. Fifteen to 20% of patients with a more severe course of illness suffer from long-lasting or permanent neuropsychiatric sequelae. In 3–11% of survivors significant neurologic morbidity is present, but TBE can also be fatal since the mortality rate is 1–2%. Patients may die in an acute stage of illness due to different causes [1, 4].

A case report of a patient from Slovenia, who died shortly after the onset of TBE, is described. The clinical course of disease, neuropathological findings, results of serological tests and polymerase chain reaction (PCR) amplification of parts of viral genome from postmortem brain tissues are presented.

Case Report

A 28-year-old previously healthy man was admitted to the Department of Infectious Diseases, University Medical Centre Ljubljana, Slovenia, on 13 May 1994 with a 3-day history of high fever, headache, vomiting, general weakness, myalgia and drowsiness. He reported tick-bites in an endemic area 1 month before the onset of symptoms. On admission he had malaise and his body temperature was 37.6°C. His mental status was normal but he was sleepy with positive meningeal signs and severe tremor. Later on the day of admission his condition rapidly deteriorated and we observed restlessness, speech and mental disorders and somnolence. The patient was transmitted to the intensive care unit (ICU). The next day the patient's condition deteriorated further, he became comatose and respiratory failure developed. Controlled mechanical ventilation was needed. The patient had continuous high fever with profound sweating. Blood pressure oscillated. He was treated symptomatically with antioedematous, antipyretic, analgetic and sedative drugs (without corticosteroids). On the fifth day of hospitalisation extremely high fever (43.2°C) developed, followed by profound hypotension, not responding to vasoactive amines. Erythrocyte sedimentation rate was 31 mm/h, and the WBC count was slightly raised (11.9 x 10^9/l) with normal differential counts. Other routine haematological and biochemical tests were normal. Lumbar puncture was performed at admission to the hospital. The CSF protein (0.45 g/l) and glucose (3.6 mmol/l, serum: 6.1 mmol/l) concentrations were normal but microscopy showed 192 x 10^6/l leukocytes (96 neutrophils and 96 lymphocytes). A serum sample tested by IMMUNOZYM-FSME Test (Behringwerke AG, Marburg, Germany) was positive for IgM TBE antibodies (optical density was up to 2.0; cutoff was 0.298), whereas ELISA IgG TBE antibody test and complement fixation test were negative. Serological tests for enteroviruses, herpes simplex virus, Epstein-Barr virus and *Borrelia burgdorferi* were negative. Cranial CT and MR were not done.

The patient died 8 days after the beginning of illness.

Neuropathological findings: An autopsy was performed and samples were taken 12h after death. On gross examination, severe brain oedema (brain weight 1,790 g) and tonsillar herniation were found. Microscopy showed focal leptomeningeal mononuclear cell infiltrates (Figure 1A), disseminated perivascular mononuclear cell cuffing in CNS (Figure 1B) and disseminated neuronophagia and glial nodules in gray matter, most prominent in medulla and anterior horns of spinal cord (Figure 1C and D). Besides, scattered perivascular microinfarcts in neocortex and diffuse mild astrogliosis of the white matter were present.

PCR amplification of TBE virus genome from postmortem brain tissue: The virological diagnosis of the TBE was additionally established by nested PCR amplification of the parts of the viral ge-

Received: 17 July 1995/Revision accepted: 25 September 1996

J. Tomažič, M. D., Ph. D., *Mojca Matičič*, M. D., *Bojana Beovič*, M. D., *Stanka Lotrič*, M. D., M. Jereb, M. D., F. Pikelj, M. D., Ph. D., Dept. of Infectious Diseases, University Medical Centre Ljubljana, Japljeva 2; *M. Poljak*, M. D., Ph. D., *Tatjana Avšič-Županc*, Ph. D., Dept. of Virology, Medical Faculty, Institute of Microbiology, University of Ljubljana, Zaloška 4; *Mara Popovič*, M. D., *Nina Gale*, M. D., Ph. D., Medical Faculty, Institute of Pathology, University of Ljubljana, Korytkova 2, SLO-1000 Ljubljana, Slovenia.

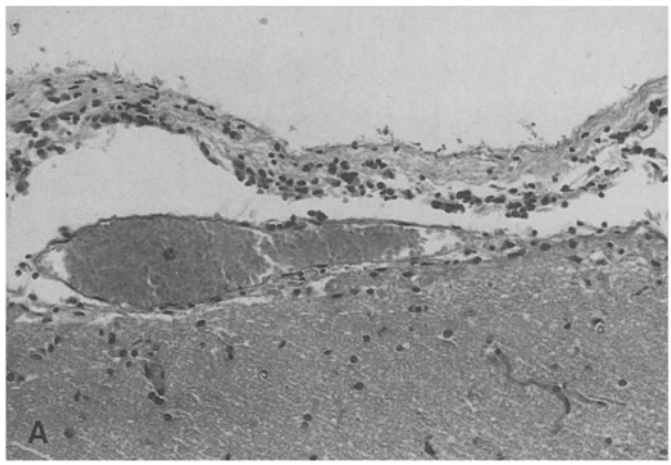

Figure 1A: Moderate mononuclear cell infiltrate and vascular congestion are present in the leptomeninges and subarachnoid space, respectively. H&E, original magnification 63.5x.

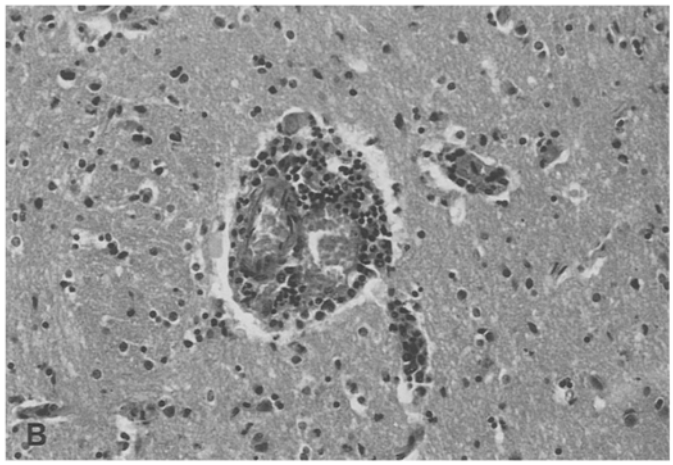

Figure 1B: Perivascular cuffing of mononuclear cells composed of activated T-cells and macrophages, H&E, original magnification 63.5x.

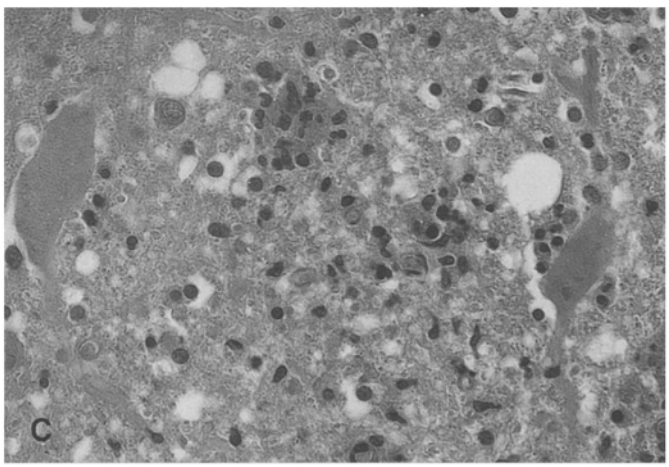

Figure 1C: Anterior horn cells are phagocytised by macrophages giving a picture of neuronophagia. Central chromatolysis is obvious in two of them confirming the incipient impairment of the pyramidal cells (arrows). H&E, original magnification 125x.

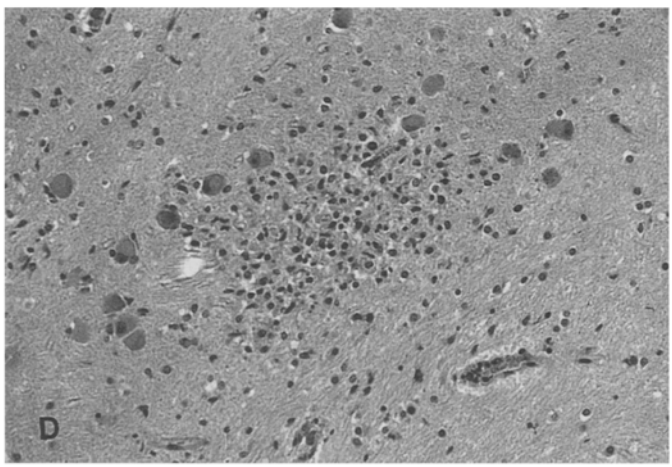

Figure 1D: Glial nodule in the inferior olivary nucleus is the fourth microscopic hall-mark of meningoencephalitis, H&E, original magnification 63.5x.

nome from the three postmortem frozen brain tissue specimens, as previously described, with minor modification (digoxigenin-11-dUTP was added to the reaction mixture in a concentration of 10 μM) [5]. Using nested PCR a 227 bp portion of TBE virus genome was amplified from all three examined postmortem brain samples. The identity of nested PCR products was determined by restriction fragment analysis using five restriction endonucleases, as previously described [5]. The restriction patterns of PCR products were compared with those of known TBE virus isolates, and found identical in all three cases, and closely related to the strain Neudoerfl, prototype of the European TBE virus subtype [6]. The identity of PCR products was additionally confirmed by enzyme-linked immunosorbent assay using a recently developed standardised PCR ELISA kit (Boehringer Mannheim, Germany) and biotin-labelled TBE group-specific probe 5'-Bio-AC-CA(AG)GAGA(AG)CCCTG designed in our laboratory. Briefly, 5–10 μl of PCR product were mixed with 40 μl of denaturing reagent (50 mM NaOH) and incubated for 10 min at room temperature. Hybridization solution containing 100 ng/ml TBE probe was then added to the denaturated PCR product to a total volume of 500 μl, gently mixed and briefly centrifuged. Two hundred microliters were transferred into microtiter wells coated with streptavidin and incubated for 3 h at 38°C on a microtiter plate shaker. After extensive washing with saline solution, 200 μl of anti-digoxigenin-horseradish peroxidase conjugate was added; this was followed by incubation at 37°C for 45 min on a microtiter plate shaker. After a second wash, 200 μl of substrate solution (1.9 mM di-ammonium 2.2'azino-di (3-ethyl-benzothiazoline-6-sulfonate) in 10 mM phosphate citrate buffer (ph 4.4) with 3.2. mM H_2O_2) was added. The color was allowed to develop for 45 min at 37°C and optical density (OD) was measured at a wavelength of 422 nm with Behring ELISA Processor II (Marburg, Germany). All three TBE nested PCR products reached OD values up to 1.5. In five TBE-negative postmortem brain tissue samples obtained from five patients (negative controls) OD values ranged from 0.021 to 0.056.

Discussion

TBE should be considered as a possibility in any patient with a clinical diagnosis of encephalitis who has been in an endemic area for TBE during May to October [7]. TBE virus as the cause of death is not frequently reported. A patient may die in the acute stage of the disease due to a severe course of the meningoencephalitic form. In severe encephalitis the mortality is due to hypoxic-ischemic brain damage and brain oedema with tonsillar herniation. When the brainstem is affected, bulbar paralysis can be expected. The involvement of vital centres (respiratory, vasoactive) also leads to life-threatening disease. Affection of the brain stem can cause extreme vegetative syndrome with cardiovascular breakdown. Patients with a life-threatening course of disease are treated in the ICU, where they are exposed to nosocomial infections and other complications due to artificial ventilation, foreign devices etc. [4].

In our case the patient presented with the typical clinical picture, lacking only a biphasic course of the disease. TBE was highly suspected on admission and quickly confirmed by specific serological testing. Treatment with anti-viral drugs was therefore omitted. High fever virtually consumed the patient and irreversible cardiovascular breakdown appeared.

As stated by *Környey*, TBE belongs to the so-called disseminated polyencephalitides with predilectional site in the brain stem [8]. Common to all of them is the lesion of periventricular regions of the brain stem, including the central nuclear group of the cerebellum, and of the reticular formation [9]. Autopsy of our patient revealed several causes of death. Extreme brain oedema with tonsillar herniation and severe inflammation of the brain stem with involvement of vital centres and affection of autonomic nervous system were found. Clinically the disease presented as the meningoencephalitic form of TBE, but neuropathological findings also revealed an affection of the anterior horns of the spinal cord, compatible with a diagnosis of meningoencephalomyelitis. If the patient had survived the acute stage, he would most probably have suffered from the paralytic form of the disease with life-long sequelae.

In conclusion, we presented the first human case in which the diagnosis of TBE was confirmed postmortem by PCR amplification of the viral TBE genome from brain tissue specimens. However, we hope that our PCR method would also be capable of direct and rapid detection of TBE RNA from whole blood samples (obtained during the first phase of the disease) or cerebrospinal fluid samples (obtained during the second phase of the disease). The testing of these possibilities is the subject of our current work.

References

1. **Kunz, C.:** Tick-borne encephalitis in Europe. Acta Leidensia 60 (1992) 1–14.
2. **Kraigher, A., Avšič-Županc, T., Kovačevič, M., Majašič, M., Matičič, M.:** Tick-borne encephalitis in Slovenia. Elipse 29 (1991) 462–463.
3. **Köck, T., Stünzer, D., Freidl, W., Pierer, K.:** Zur Klinik der Frühsommermeningoenzephalitis (FSME) in der Steiermark. Nervenarzt 63 (1992) 205–208.
4. **Pikelj, F., Tomažič, J., Matičič, M., Sočan, M., Muzlovič, I.:** Severe forms of tick-borne meningoencephalitis in Slovenia. J. Infect. 31 (1995) 83–85.
5. **Avšič-Županc, T., Poljak, M., Matičič, M., Radšel-Medveščcek, A., LeDuc, J. W., Stasny, K., Kunz, C., Heinz, F. X.:** Laboratory acquired tick-borne meningoencephalitis: characterisation of virus strains. Clin. Diagn. Virol. 4 (1995) 51–59.
6. **Mandl, C. W., Heinz, F. X., Stoeckl, E., Kunz, C.:** Genome sequence of tick-borne encephalitis virus (western subtype) and comparative analysis of nonstructural proteins with other flaviviruses. Virology 173 (1989) 291–301.
7. **McNair, A. N. B., Brown, J. L.:** Tick-borne encephalitis complicated by monoplegia and sensorineural deafness. J. Infect. 22 (1990) 81–86.
8. **Környey, S.:** Zur vergleichenden Pathologie der Zeckenencephalitiden. Verh. Dtsch. Ges. Inn. Med. 61 (1955) 231–235.
9. **Környey, S.:** Contribution to the histology of tick-borne encephalitis. Acta Neuropathol. 43 (1978) 179–183.