Fatal Encephalitis Caused by Concomitant Infection with Tick-Borne Encephalitis Virus and *Borrelia burgdorferi*

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We describe a 38-year-old farmer from the southwestern archipelago of Finland where both tick-borne encephalitis (TBE) virus and *Borrelia burgdorferi* are endemic. He presented with fever and headache, developed severe meningoencephalitis in 3 days, and, after 1 month, died without regaining consciousness. High titers of IgG and IgM antibodies to TBE virus were present in both serum and CSF. Serology for *Borrelia* was negative. Autopsy revealed necrotizing encephalitis and myelitis with involvement of the dorsal root ganglion. With use of polymerase chain reaction tests, segments of two separate genes of *B. burgdorferi* were amplified from the patient's CSF. This case demonstrates that the possibility of dual infection should be considered for patients residing in geographic areas where *Ixodes* ticks may carry both the TBE virus and *B. burgdorferi*. We believe that the most severe damage in this case was caused by TBE virus rather than by *B. burgdorferi*. Nevertheless, the coinfection might have contributed to the fatal outcome that has not been previously observed in Finnish patients with TBE.

The causative spirochete of Lyme disease, *Borrelia burgdorferi*, and the virus of tick-borne encephalitis (TBE) are both transmitted by certain *Ixodes* ticks [1, 2]. *Ixodes ricinus* is the main vector in central Europe. In the United States, the main vector of Lyme borreliosis is *Ixodes dammini*, which can also transmit a flavivirus related to the viruses in central Europe and Finland [1–3]. In Finland, the main vector of TBE is *I. ricinus*. The TBE virus in Finland is called Kumlinge virus and is so named because it was found on the island of Kumlinge in the Finnish archipelago. It is a flavivirus identical with the central European TBE virus [1].

Lyme borreliosis may affect the skin, heart, joints, and nervous system. Neurological manifestations include meningitis, cranial neuritis, motor or sensory radiculoneuritis, polyneuropathy, chronic encephalopathy, and meningoencephalitis [4]. Recently, an expansive cerebral lesion has, moreover, also been reported [5]. Chronic encephalopathy and meningoencephalitis are late manifestations of the disease [4]. This late stage mimicks chronic infection with *Treponema pallidum*, usually commencing ≥1 year after the onset of disease. Late sequelae of borreliosis can be prevented by appropriate treatment of the acute infection.

TBE is often a subclinical infection. It may, however, cause severe encephalitic symptoms, including permanent paresis. There is no specific treatment, but the disease can be prevented by immunization [1, 6].

We report here a concomitant infection by the TBE virus and *B. burgdorferi* that caused severe encephalomyelitis with a fatal outcome.

Case Report

The patient was a 38-year-old previously healthy farmer and hunter from the southwestern archipelago of Finland. In winter, in spring, and again in June 1990, he experienced episodes of fever and symptoms of upper respiratory tract infection. Each episode lasted for about 3 weeks. He received antimicrobial therapy for every episode; however, there is no information available regarding the antibiotics used or the duration of the treatments. The patient recalled having had several tick bites earlier during his lifetime but not at the time immediately before the development of symptoms, neither had he recently suffered from erythema migrans.

On 17 September 1990, the patient became febrile and was admitted to a local hospital 2 days later. On admission, he had fever, chills, and moderate headache but no signs of meningeal irritation. Slight pharyngitis was found, and enlarged lymph nodes were palpable in the neck, supraclavicular space, and right axilla. The chest roentgenogram revealed no abnormalities. The erythrocyte sedimentation rate was 13 mm/h, and the leukocyte count was 6.4×10^9/L, with 19% band forms. The level of serum C-reactive protein (CRP) was elevated to 170 mg/L (normal level, <10 mg/L). On the basis of these findings, a presumptive diagnosis of septicemia was made.

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Table 1. Dates and results of analysis and antibody tests of CSF and serum.

Test (unit)	Test results for indicated date				
	9/19/90	9/24/90	9/28/90	10/2/90	10/5/90
CSF					
Leukocyte count ($\times 10^6$/L)	61	870		10	6
Proportion of neutrophils (%)	32	48		5	0
Lactic acid concentration (mmol/L)*		10.6		3.5	
Protein concentration (mg/L)†	254	1,700		602	
Titer of antibodies to TBE virus‡					
IgM		Positive		Positive	Positive
IgG		1:256		1:256	1:256
Titer of antibodies to *Borrelia burgdorferi*				Negative	
PCR amplification of *B. burgdorferi* DNA				Positive	
Serum					
Titer of antibodies to TBE virus					
IgM			Positive	Positive	
IgG			1:10,240	1:5,120	
Titer of antibodies to *B. burgdorferi*	Negative		Negative		

* Normal value, <2.7 mmol/L.
† Normal value, <495 mg/L.
‡ IgG antibodies in CSF were negative when <1:4.

Therapy with penicillin G (4 million IU iv every 4 hours) and gentamicin (80 mg iv every 8 hours) was started. The next day, therapy with penicillin G was changed to that with cefotaxime (2 g iv every 6 hours) because of suspected urinary tract infection.

On the third day of high fever, the patient developed meningismus and became confused. The CSF contained 61 $\times$ 10^6 leukocytes/L, with 32% neutrophils. Cultures of blood and CSF were repeatedly negative. The antibacterial treatment was discontinued, and therapy with acyclovir (750 mg iv every 8 hours) for putative herpes simplex virus encephalitis was instituted.

On September 24, the patient was transferred to Turku University Hospital. In the emergency department, he became comatose, had convulsions, and received respiratory support. There were signs of extensive meningeal irritation. Analysis of CSF showed pleocytosis and increased concentrations of lactic acid and protein (table 1). Gram stain, cultures, and tests for bacterial antigens (*Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae,* group B *Streptococcus,* and *Escherichia coli*) were all negative. The serum CRP level was 186 mg/L, gelation of plasma ethanol occurred, and levels of products of fibrin degradation were slightly elevated. Concentrations of serum immunoglobulins were at normal levels.

On neurological examination, extension of the extremities was the only reaction to pain. Electroencephalography showed an irritative focus on the left side. Findings on a computed tomographic scan of the brain were normal. The working diagnosis was meningitis or encephalitis of bacterial origin.

Therapy with cefotaxime (2 g iv every 6 hours) was reinstituted, and 4 days later erythromycin (1 g iv every 6 hours) and gentamicin (80 mg iv every 8 hours) were added to the therapeutic regimen. Dexamethasone (4 mg iv every 6 hours) and diphenylhydantoin (first 250 mg and then later 125 mg iv every 12 hours) were given to reduce cerebral edema and to prevent convulsions.

On October 2, the patient's clinical status was unchanged, even though the CSF analysis showed clear improvement (table 1). At this stage, a diagnosis of TBE was confirmed. Antibodies to TBE (Kumlinge) virus were measured by enzyme immunoassay with use of formalin-inactivated purified TBE virus as an antigen. Levels of IgG antibodies were strongly elevated in both serum and CSF. Furthermore, IgM antibodies to the virus were detectable in both serum and CSF, thus suggesting a recent infection (table 1). No increase in levels of antibodies to any other tested viruses was observed, and the serology for human immunodeficiency virus was negative. With use of ELISA, significantly raised levels of antibodies to sonicated whole *B. burgdorferi* and to purified endoflagellar antigen were not detected in either serum or CSF (table 1). A CSF specimen for polymerase chain reaction (PCR) for *Borrelia* was taken. Since the TBE virus infection was confirmed, therapy with antimicrobial agents and dexamethasone was discontinued. Although the patient remained unconscious, he slowly became afebrile, underwent tracheostomy, and was able to breathe spontaneously.

On October 9, the patient experienced a new episode of high fever (temperature, 40°C). The CRP level rose from <10 to 201 mg/L. The chest roentgenogram showed parenchymal infiltration, indicating pneumonia. Antibiotic ther-

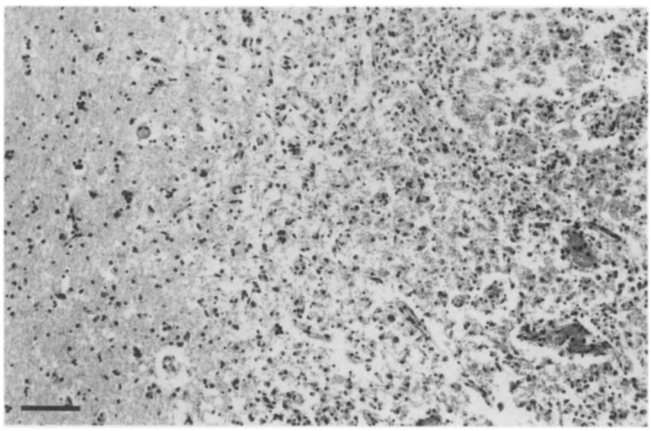

Figure 1. The pyramidal layer of the hippocampus is destroyed by hemorrhagic necrosis. In addition to erythrocytes, there are numerous foamy macrophages phagocytosing the debris (stain, hematoxylin-eosin; bar = 100 μm).

apy with imipenem (1 g iv every 8 hours), rifampicin (300 mg iv every 12 hours), and vancomycin (500 mg iv every 6 hours) was started. Two days later, amphotericin B (80 mg iv daily) was added to the therapeutic regimen. Imipenem-sensitive *E. coli* was isolated from the trachea. Blood cultures were repeatedly negative, as were all cultures of specimens from the cannulas. Later, *Clostridium difficile* was isolated from a stool sample obtained while the patient had diarrhea. On October 13, the patient died.

With use of the PCR method, a *B. burgdorferi*–specific segment of a gene coding for a 41-kD endoflagellin [7] and a *Borrelia*-specific segment of 16S rDNA [8] were amplified from the CSF (table 1). The primers and temperatures were exactly the same as those in the original publications. The identity of the amplified product obtained from the endoflagellin gene was further confirmed by positive reactivity with a probe specific for *B. burgdorferi*. The sequence of the oligonucleotide probe was as described earlier [9].

Postmortem findings. The autopsy revealed pulmonary edema and active bronchitis but no clear signs of pneumonia. The spleen, liver, and pancreas were moderately congested. No signs of pancreatitis or pseudomembranous colitis were observed.

The brain was moderately edematous with signs of mild tentorial herniation. Mild meningitis was verified by diffuse lymphocytic infiltrates in the subarachnoid space. Extensive, partly hemorrhagic necrosis was seen in the pyramidal cell layers of both hippocampi (figure 1). Smaller necrotic foci were present in the cerebral cortex, most conspicuously in the right temporal lobe. In the thalamus, a marked loss of neurons with focal neuronophagia was observed; diffuse and nodular astrocytic and microglial reaction and prominent perivascular lymphocytic infiltrates were evident. Moreover, a loss of neurons and encephalitic microglial nodules was detected in the amygdaloid nuclei, in the cerebellum, in the anterior horn of the spinal cord, in the dorsal root ganglia, and, to a lesser extent, in the brain stem. Consistent with ganglionic and spinal involvement, wallerian degeneration was present in the spinal roots. In the frontal white matter, there were small perivascular foci of myelin destruction associated with an accumulation of macrophages laden with myelin debris. Seven brain-tissue blocks with encephalitic changes were examined immunocytochemically with use of a hyperimmune rabbit antiserum to TBE virus and visualized by the peroxidase-antiperoxidase technique. The results were negative, while a strong positive reaction was seen in identically processed, experimentally infected mouse brain.

Cultivation of *Borrelia* from the brain tissue was not performed. PCR analyses of formaldehyde-fixed brain tissue from the hippocampus and thalamus were negative for *Borrelia*. Likewise, in situ hybridization of paraffin-embedded brain tissue from areas with tissue necrosis and/or inflammatory cell infiltrates with use of the above-mentioned probe for *B. burgdorferi* was negative.

Discussion

To our knowledge, this is the first fatal case of TBE reported in Finland. Among the earlier described 126 patients suffering from TBE, no deaths occurred [1]. The most severe cases, until now, involved two patients with permanent paresis of the upper limb and five patients with transient paresis (including facial paresis, eye muscle paresis, and left hemiparesis) [1]. Reports of fatal cases elsewhere have also been rather rare. For example, in Sweden, as few as five deaths have been reported to occur among 1,091 cases, and in West Germany, only three deaths have been reported among 299 cases [6, 10, 11]. In the Soviet Union, TBE has been reported to be associated with a much higher mortality rate: 6.4% in the meningeal and up to 33% in the focal encephalitic forms of the disease [12].

There is at least one case report in the literature of a double infection with TBE virus and *B. burgdorferi* [13]. In that patient, multiple peripheral pareses occurred in the left leg 3 weeks after the onset of neurological symptoms and after recovering from encephalitis.

In our report of double infection, the diagnosis of TBE was verified by strongly positive serology and compatible brain pathology. The diagnosis of neuroborreliosis was based on positive results of PCR of the patient's CSF. Because of the extreme sensitivity of PCR methodology, several precautions must be exercised with regard to assay performance and interpretation. In our study, all measures for avoiding contamination were strictly adhered to in assessing and reassessing the specimens. Specimens from other patients that were assayed simultaneously were negative, as was also the negative control, containing all other PCR reagents except the sample DNA. Thus, the result of the PCR test can be considered definite proof of borreliosis in our patient, despite the sero-

negativity. It has previously been suggested that a minority of patients who suffer from late-stage borreliosis remain seronegative, particularly if they have received inappropriate antibiotic treatment during early stages of their disease [14]. Although respiratory failure appeared to be the immediate cause of death of our patient, brain pathology was, in fact, significant enough to be related to the fatal outcome.

The neuropathology of Lyme borreliosis is poorly known. One detailed neuropathological study of the CNS in a case of tertiary Lyme neuroborreliosis showed some meningovascular findings: chronic meningitis as the essential lesion leading to Heubner's specific endarteritis, with resultant occlusive thrombosis and secondary cerebral infarcts. Direct invasion of the parenchyma of the CNS by the spirochetes is another possibility for parenchymal brain involvement [15]. Millner et al. [16] described a child suffering from arthritis who died during protracted grand mal epileptic seizures, the first neurological sign of her disease: general vasculitis and *B. burgdorferi* spirochetes were demonstrated at autopsy [16]. The most common form of chronic neurological abnormalities that affect the CNS in patients with borreliosis has been subacute encephalopathy—in which the pathological process is multifocal and generalized, affecting both gray and white matter [4].

It is known that the TBE virus can cause meningitis or a more severe infection of the gray matter of the CNS (i.e., polioencephalitis and polioencephalomyelitis) associated with cerebral edema [1, 10, 11]. The predilectional sites of viral lesions are evidently deep within the cerebral gray matter (especially the thalamus), periventricular regions of the brain stem, cerebellar cortex, and anterior horns of the spinal cord [17]. We found two main types of tissue damage. First, inflammatory changes and nerve cell loss involved deep areas of gray matter. These changes are compatible with subacute TBE virus infection, with regard to both quality and distribution of the lesions [17]. Together with serology, these changes confirmed the diagnosis of TBE. Second, in the hippocampus and cerebral cortex, we found laminar necroses similar to hypoxic-ischemic brain injury. Similar findings are reported for TBE neuropathology [17], but localized vasculitis [16] due to borreliosis is another possible explanation for these.

Negative immunocytochemical results for TBE virus do not disprove TBE. Viral antigen in the brain has been found only during the acute phase of experimental infection and has been detected in the brains of none of 12 patients with serology-proven disease. The clinical course of all these patients with TBE was >10 days (H. Budka, unpublished observation). Apparently, flavivirus antigen is soon cleared from, or masked in, the human brain after the acute stage of encephalitis. In a report of another flavivirus infection, Japanese B encephalitis, viral antigen in the brain was progressively cleared in patients who survived ≥6 days [18]. Since the neuropathological findings in our case are very similar to earlier reported findings in TBE cases, we believe that the causative agent of severe encephalitis as described was the TBE virus rather than *B. burgdorferi*.

The findings in this report indicate that our patient was infected with both the TBE virus and *Borrelia*. Both of the organisms as well as the vector *I. ricinus* are endemic in the Finnish archipelago, where the patient was living. As many as 85% of the local population recall one or more tick bites in their lifetime, and 16% of them have had erythema around the tick bite [19]. Furthermore, as a farmer and active hunter, our patient was in very intimate contact with the habitat of the tick. It is also evident that some of these ticks can harbor both microbes simultaneously. Thus, our patient may have contracted both of the organisms from a single tick bite, with both the resulting infections being of an acute nature. TBE usually evolves in two or a few weeks [10, 20], whereas encephalitic symptoms occur during the late stages of borreliosis months or even years after the primary infection [4, 21, 22]. However, rapid invasion of the CNS by *B. burgdorferi* has also been documented [23]. The seronegativity of the patient favors the presence of acute borreliosis. The predominantly granulocytic pleocytosis, responsive to antibiotic treatment, supports active borrelia infection. The dexamethasone therapy might have weakened the efficacy of antimicrobial treatment against *Borrelia* [15, 22].

This case report emphasizes that dual infections should be taken into account in localities where both the TBE virus and *B. burgdorferi* are endemic and prevalent. Because borreliosis can be treated, its early diagnosis is of utmost importance. Problematic, in this regard, is that confirmation of borreliosis by cultivation may take several weeks. Moreover, serology may remain negative, as in this case, even in the later stages of the disease. We submit that PCR has opened new perspectives in the rapid diagnosis of many infectious diseases. Specifically, it provided the diagnosis of borreliosis in this case.

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