Serologically Proven Double Infection with Tick-Borne Encephalitis Virus (TBEV) and *Borrelia burgdorferi**

Serologisch gesicherte Doppelinfektion durch Frühsommer-Meningoenzephalitis (FSME-) Virus und *Borrelia burgdorferi*

K.-P. Hunfeld[1,4], Regina Allwinn[2], Sabine Albert[1], Petra-Maria Schumm-Draeger[3], V. Brade[1], H.W. Doerr[2]

Summary: Tick-borne encephalitis virus (TBEV) and *Borrelia burgdorferi* remain the clinically most relevant agents transmitted by ticks in European countries. Our report demonstrates a serologically proven TBEV infection despite passive immunoglobulin prophylaxis going hand-in-hand with a subclinical course of borreliosis in a 69 year old German female patient suffering from somnolence and diplopia after a tick bite in Austria. Definitive diagnosis was achieved by serological investigations revealing specific IgG and IgM antibodies for TBEV and *Borrelia burgdorferi* in ELISA and recombinant immunoblotting, respectively. The appearance of concomitant or double infections with TBEV and *Borrelia burgdorferi* is exceptional but coinfection due to both agents should be considered in the differential diagnosis of tick-borne disease, especially if the clinical presentation is unusual.

Keywords: Tick-Born Diseases; Encephalitis Viruses, Tick-Borne: Lyme Disease; Borrelia burgdorferi.

Zusammenfassung: Das Virus der Frühsommermeningoenzephalitis (FSME) und *Borrelia burgdorferi* als Erreger der Lyme-Borreliose sind die klinisch bedeutsamsten durch Zecken übertragenen Infektionserreger in Europa. Der vorliegende Fall beschreibt eine serologisch gesicherte Doppelinfektion mit dem FSME-Virus und *Borellia burgdorferi* bei einer 69jährigen deutschen Patientin nach einem Zeckenstich in einem österreichischen Endemiegebiet. Klinisch bestand zum Zeitpunkt der Krankenhausaufnahme eine ausgeprägte Somnolenz und ein hochgradiges Doppelbildsehen. Ein passive Immunisierung gegen FSME war postexpositionell erfolgt, konnte eine Infektion jedoch nicht verhindern. Eine Doppelinfektion durch beide Erreger wurde durch den serologischen Nachweis von spezifischen IgG und IgM Antikörpern gegen das FSME-Virus und im weiteren Verlauf auch gegen *Borrelia burgdorferi* im ELISA beziehungsweise im rekombinanten Immunoblot gesichert. Obwohl Doppelinfektionen durch die beiden genannten Erreger selten sind, sollten sie bei zeckenübertragenen Erkrankungen mit untypischem Verlauf in der Differentialdiagnose berücksichtigt werden.

Schlüsselwörter: Zeckenübertragene Erkrankungen; Frühsommermeningoenzephalitis; FSME-Virus, Lyme-Borreliose, Borrelia burgdorferi.

Microbial organisms transmitted by ticks represent a significant part of new emerging infectious agents. A possible explanation for biological variation of tick transmitted diseases might be unapparent coinfection with other pathogens. So far, little is known on the ecological behavior of concurrent pathogens within their vectors and the possible consequences with regard to infections of the human host. Seroepidemiological findings suggest that coinfections with more than one organism may occur after tick bite [1, 2, 3]. However, only few reports could demonstrate this phenomenon in clinically ill patients. In these cases the influence of concomitant infections on the severity and the clinical outcome of the disease have to be investigated. We report on a serologically proven coinfection due to tick-borne encephalitis virus (TBEV) and *Borrelia burgdorferi* in a 69 year old woman. To our knowledge only four other cases have been reported in the scientific literature [4, 5, 6, 7].

Methods

FSME complement fixation test (CFT)

CFT for FSME [Virion, GmbH, Germany] [8] was performed in accordance with the manufacturers instructions. A serum with a specific antibody titer of $\geq 1:5$ was considered positive, whereas a titer of $< 1:5$ was considered negative.

*This report is dedicated to Prof. Dr. med. *R. Bässler* for his 72th birthday.

[1]Institut für Medizinische Mikrobiologie,
[2]Institut für Medizinische Virologie,
[3]Medizinische Klinik I, Zentrum der Inneren Medizin, Universitätsklinikum Frankfurt/Main.
[4]Correspondence to: Dr. med. K.-P. Hunfeld, Institut für Medizinische Mikrobiologie, Universitätsklinikum Frankfurt/Main, Theodor-Stern-Kai 7, D-60590 Frankfurt/Main. Fax: +49-69-6301-5767, E-mail: K.Hunfeld @em.uni-frankfurt.de
Received: April 4, 1998 / Accepted: June 2, 1998

ELISA-testing

To determine antibody titers against TBEV and *Borrelia burgdorferi*, assays were performed separately for IgG and IgM using commercial ELISA kits (Immunozym-FSME-ELISA, Immuno GmbH, Germany, Enzygnost Borreliosis, Dade Behring, Germany) [9, 10]. For detection of specific IgM the serum samples were preincubated with rheumatoid factor absorbance reagent. The sample diluent of the borreliosis ELISA contained ultrasonicated cell lysates of *Treponema phagedenis* for absorption of cross-reactive antibodies. Samples and controls were read on an ELISA reader (MR 7000, Dynex, Germany) at 450 nm. Cut-off values and qualitative test results for IgM and IgG were calculated from the optical density (OD) as stated by the manufacturer. Quantitative testing for TBEV specific IgG was determined in Vienna units (VIEU). Results were classified as positive (>126 VIEU), borderline (63-126 VIEU) and negative (<63 VIEU). Quantitative test results for *Borrelia burgdorferi* specific IgG were determined in units / ml in accordance with the manufacturer's instruction. A serum with an activity of > 10 U/ml was considered positive. Determinations used to assess significant changes in activity were performed in the same run and in the same test dilution. A difference of more than a factor of 2 was regarded as indicative of a significant change in serum activity.

Recombinant immunoblot

The IgG and IgM immune responses were determined separately in each serum sample using a commercially available recombinant immunoblot (Figure 1, Recomblot *Borrelia burgdorferi*, Microgen, Germany) with highly specific *Borrelia burgdorferi* antigens [p83/p100, flagellin (p41), outer surface protein C (OspC), outer surface protein A (OspA), flagellin, internal fragment (p41i); (Figure 1)] [11, 12, 13]. To avoid false positive results in detecting IgM antibodies, serum samples were preincubated with rheumatoid factor absorbance reagent (Mastsorb, Mast Diagnostica, Germany) [13]. Interpretation of test results was carried out by an evaluation of specific antigens (bands) provided by the manufacturer on the basis of clinical testing and a mathematical analysis. The IgM immunoblot was interpreted as positive (presence of OspC or several other bands), equivocal (either presence of p41 and p41i or presence of only one of the following bands: p83/p100, OspA, p41 or p41i) or negative (absence of specific reactivity). The IgG immunoblot was classified as positive (presence of p100 and/or OspC and several other bands), equivocal (presence of p41 and p41i or presence of only one of the following bands: p83/p100, OspA or OspC) or negative (absence of specific reactivity). To discriminate between positive, borderline and background reactivity of specific bands predefined weak positive controls were provided by the manufacturer and served as a cut-off. Determinations used to assess significant changes in serum reactivity were performed in the same run and in the same test dilution.

Clinical and laboratory findings

A 69 year old female patient suffering from chronic fatigue, somnolence and diplopia was admitted to the department of internal medicine of the University Clinic, Frankfurt in August 1995. She remembered an episode of flue-like illness two weeks before with fever and moderate headache lasting for a few days. The patient recalled a tick bite during a holiday trip to Austria at the end of July 1995, with subsequent passive immunization for TBEV (0.2 ml/kg FSME-immunglobulin-Behring, Dade Behring, Germany) within 24 hours. No erythema migrans or further manifestations of Lyme-disease had been noted since. Former medical history laid open resection of the thyroid gland because of Graves disease 25 years ago followed by chronic recurrent endocrine orbitopathy. On admission physical examination showed diplopia and a slightly decreased vision of both eyes, but no signs of meningeal irritation were found. Laboratory findings and the chest radiographs revealed no abnormalities, body temperature was not elevated. As the patient obviously lacked signs of local or systemic infection the presumptive diagnosis of a recurrent episode of endocrine orbitopathy was made based on the clinical findings. While ophtalmological examination and magnetic resonance imaging showed no hints for acute worsening of the existing orbitopathic symptoms, subsequent serological investigations revealed positive IgM and IgG antibody titers for FSME in ELISA and a positive CFT. In October 1995 examination of a follow-up serum sample confirmed these findings by demonstrating TBEV specific IgM and IgG antibodies and a significant (1:5 versus 1:40) increase of TBEV specific CFT titer (Table 1). Serological investigation for Lyme borreliosis remained negative in August 1995. Antibody reactivity was positive for Cytomegalovirus IgG, Epstein-Barr-Virus IgG and Herpes-simplex virus IgG, but proved negative for the corresponding IgM antibodies. The clinical findings were thus interpreted as a mild manifestation of TBEV after previous passive immunization against this agent.

The patient's condition improved quickly under symptomatic treatment. She refused a lumbar puncture to confirm intrathecal antibody production and was discharged a few days later.

In October 1995 serological follow up showed IgG antibody reactivity against *Borrelia burgdorferi* in ELISA, showing significant increase (negative versus 300 U/ml) and decrease (300 U/ml versus 50 U/ml) of serum activity (Table 1). Coinfection with *Borrelia burgdorferi* was confirmed by positive immunoblotting for IgG and IgM using highly specific recombinant proteins (Figure 1). Serological screening for *Trepone-*

Non standard abbreviations: CFT, complement fixation tests; ELISA, enzyme-linked immunosorbent assay; OD, optical density; OspA, outer surface protein A; OspC, outer surface protein C; TBEV, Tick-borne encephalitis virus; TPHA, *treponema pallidum* hemagglutination assay; VIEU, Vienna units.

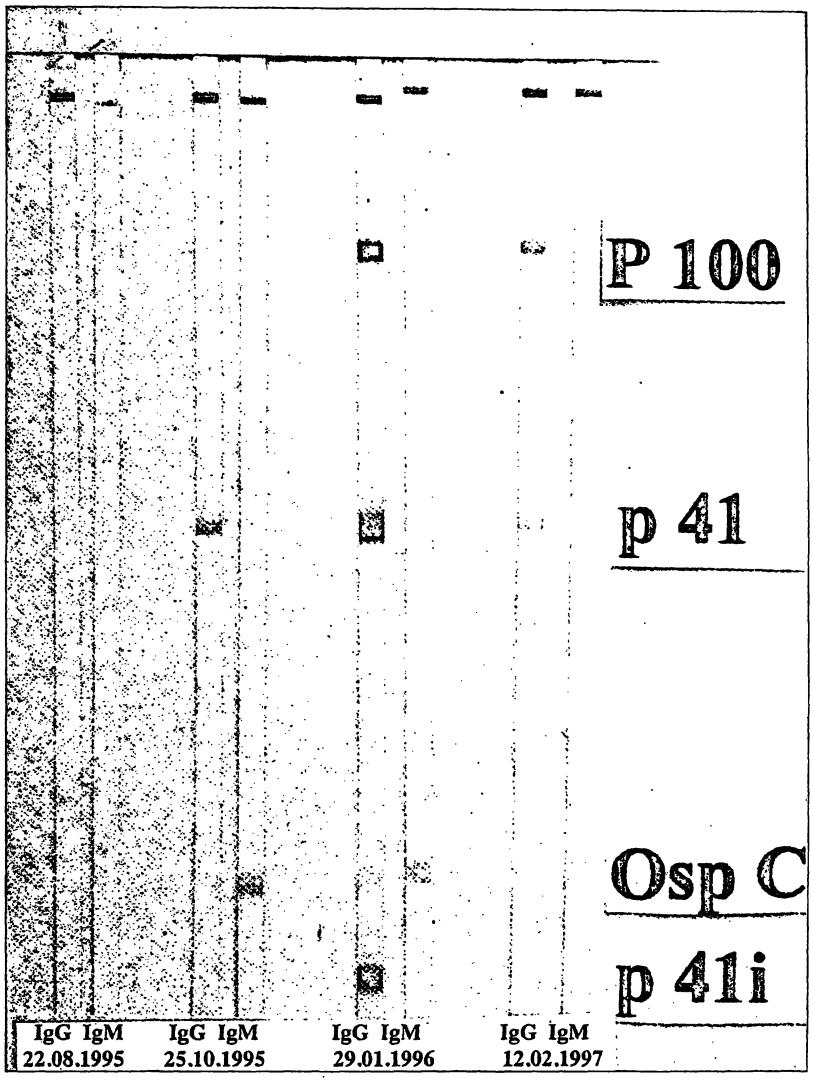

Figure 1 Cumulative results of immunoblotting with recombinant *Borrelia burgdorferi* antigens [p83/p100, flagellin (p41), outer surface protein C (OspC), outer surface protein A (OspA), flagellin, internal fragment (p41i)]. An increase of specific bands is seen during serological follow-up, reflecting the serological course of a current infection.

ma pallidum infection by TPHA (Mast-TPHA-Lues-Test, Mast Diagnostica, Germany) proved negative.

In February 1996 no more TBEV-specific IgM could be detected, while IgG- and IgM-antibodies for *Borrelia burgdorferi* were still present in the serological examination. The serological findings of the whole follow-up period are summarized in Table 1 and Figure 1. The patient showed no signs of manifest Lyme disease neither at the time of the first serological diagnosis nor during the 1½ year follow-up period and therefore no antibiotic treatment was instituted.

Discussion

In central Europe besides transmitting *Borrelia burgdorferi*, the causative spirochete of Lyme-disease *Ixodes ricinus*,, is also known as the main vector of TBEV, a virus belonging to the widely distributed family of the flaviviridae [14]. Both organisms represent the clinically most relevant agents transmitted by ticks in the European countries which together are estimated to infect some 10 000 people a year [15, 16, 17]. While *Borrelia burgdorferi* shows a more general distribution all over Europe, the occurrence of TBEV is epidemiologically correlated to certain endemic foci [15, 16, 17]. In their study dealing with the seroprevalence of TBEV and Lyme-borreliosis in a defined Swedish population *Gustavson* et al (1990) found antibody reactivity to both tick-transmitted pathogens in sera from 14 out of 346 healthy donors (4%) [1].

The theory of possible concomitant infections by more than one agent after a tick bite is further supported by the 20% positivity rate for antibodies to both

Table 1 Clinical data and cumulative serological findings

Date	Clinical data	TBEV-CFT (pos : ≥1:5)	TBEV ELISA-IgG (pos. >126 mE/ml	TBEV-ELISA-IgM	Borrelia-ELISA-IgG (pos > 10 U)	Borrelia-ELISA-IgM	Recombinant Borrelia-Immunoblot (IgG)	Recombinant Borrelia-Immunoblot (IgM)
July 95	holiday trip to Austria, tick bite, subsequent passive immunoglobulin prophylaxis	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
08.08.95	flue-like illness	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
21.08.95	somnolence, diplopia, hospital admission	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22.08.95	diagnosis of TBEV infection	+ (1:5)	+ (319 mE/ml)	+	neg	-	neg	neg
25.10.95	diagnosis of *Borrelia burgdorferi* infection	+ (1:40)	+ (433 mE/ml)	+	+/- (10)	-	p41	OspC
29.01.96	asymptomatic	+ (1 :5)	n.d.	n.d.	+ (190)	-	p100, OspC, p41, p41i	OspC
26.02.96	asymptomatic	n.d.	+ (394 mE/ml)	-	+ (210)	-	n.d.	n.d.
04.03.96	asymptomatic	n.d.	n.d.	n.d.	+ (300)	-	p100, OspC, p41, p41i	OspC, p100
09.12.96	asymptomatic	n.d.	+ (381 mE/ml)	-	+ (70)	-	p100, p41, p41i	OspC
12.02.97	asymptomatic	n.d.	+ (365 mE/ml)	-	+ (50)	-	p100, p41	OspC

Legend: +: positive, n.d.: not done, -/neg: negative. Specific proteins of *Borrelia burgdorferi:* p83/1p00, outer surface protein C (OspC), flagellin (p41), flagellin, internal fragment (p41i)

Borrelia burgdorferi and the newly discovered *Ehrlichia species* causing human granulocytic ehrlichiosis (HGE) in certain endemic regions [2]. Likewise, *Mitchell* et al (1996) demonstrated serological evidence for coinfection with another organism, either *Borrelia burgdorferi* or *Babesia microti*, the causative agent of human babesiosis in 15% of their HGE patients [3]. During the last decade only four cases of coinfections with TBEV and *Borrelia burgdorferi* have been reported [4, 5, 6, 7]. All patients showed an unusual presentation of the primarily diagnosed tick-borne infection leading to further laboratory investigations that finally confirmed the contribution of more than one tick-transmitted pathogen to the clinically atypical manifestation of the disease. One patient even died from a coinfection caused by both agents with subsequent fatal encephalitis [6]. Nevertheless, as seen in our case, one or both infections may show a more or less subclinical or an asymptomatic course of the illness, which the patient is frequently not even aware of.

In the present case a current TBEV infection was confirmed by positive TBEV-IgG antibody testing and a significant increase of TBEV-CFT beside corresponding positive TBEV-specific IgM titers over an 8 week period which cannot be explained by preceding passive immunization. Besides fever, the appearance of diplopia and somnolence is not uncommon in tick-borne encephalitis because of the affinity of the virus to the cerebellum and the brain stem [19, 20]. Our patient did not suffer from fever. This is not inconsistent with the diagnosis of TBEV, because up to 39.5% of the patients with meningoencephalitic or menigoencephalomyelitic forms of TBE may show a body temperature that is normal or below 38 °C [19].

A coinfection with *Borrelia burgdorferi* was proved 8 weeks later by a significant increase of specific antibody reactivity in ELISA and immunoblotting. False positive test results due to antigenic cross-reactivity between both agents have not yet been described in patients suspected of having either TBEV or Lyme

borreliosis. Acute Lyme disease occurs 1 - 4 weeks after an infection with *Borrelia burgdorferi* and may present with unspecific symptoms such as somnolence and fatigue, but not with diplopia [18]. In general neurological symptoms do not appear within two weeks after a tick bite [18]. Hence, the latter symptom of our patient was probably not a sign of borreliosis and we therefore consider the clinical presentation of the present case to be due to TBEV manifestation. About 50% of patients infected with TBEV or *Borrelia burgdorferi* do not remember a tick bite. Consequently, the probability of a second tick bite which was not noted is the same as the probability of a concomitant infection by both agents due to a single tick bite.

According to recent investigations passive immunization for TBEV within 48 hours after tick bite prevents an infection in only 40-50% of the cases [21]. In some patients neurological adverse reactions or particularly severe forms of disease are observed [22]. In the present case passive immunization may have contributed to the less severe clinical outcome.

Summing up, our report supports the existence of coinfections by tick-transmitted agents, even if the risk may be low. Hence, concomitant borreliosis should be considered in the differential diagnosis of tick-born encephalitis, especially when the clinical presentation is unusual.

References

1. Gustavson R, Svenungsson B, Gardulf A, Stiernstedt G, Forsgren, M. Prevalence of tick-borne encephalitis and Lyme-Borreliosis in a defined Swedish population. Scand Infect Dis 1990;22:297-306.
2. Wong SJ, Brady GS, Dumler JS. Serological response to *Ehrlichia equi, Ehrlichia chaffeensis* and *Borrelia burgdorferi* in patients from New York State. J Clin Microbiol 1997;35:2198-2205.
3. Mitchell, PD, Reed, KD, Hofkes, JM. Immunoserologic evidence of coinfection with *Borrelia burgdorferi, Babesia microti* and human granulocytic *Ehrlichia* species in residents of Wisconsin and Minnesota. J Infect Dis 1996;34:724-7.
4. Kristoferitsch W, Stanek G, Kunz C. Doppelinfektion mit Frühsommermeningoenzephalitis (FSME-) Virus und *Borrelia burgdorferi*. Deutsch Med Wschr 1986;111:861-4.
5. Abtshagen R, Bahr J. Doppelinfektion *Borrelia burgdorferi* - FSME Virus. Kinderärztl Praxis 1992;60:103 (letter).
6. Oksi J, Viljanen MK, Kalimo H, Peltonen R, Marttila R, Salomaa P, Nikoskelainen J, Budka H, Halonen P. Fatal encephalitis caused by concomitant infection with tick-borne encephalitis virus and *Borrelia burgdorferi*. Clin Infect Dis 1993;16:192-6.
7. Bobrowska E, Grezeszcuk A, Flisiak R. Tick-born encephalitis and concomitant infection with *Borrelia burgdorferi*. Rocz Akad Med Bialymst 1996;41: 40-50.
8. Höhne-Reichel G, Doerr HW. Die Komplementbindungsreaktion (KBR) in der Virusserologie: Vergleich mit modernen Immunoassays an ausgewählten Fallbeispielen. Lab Med 1984;30: 133-40.
9. Kießing ST, Abel U, Risse P, Friedrich J, Heinz FX, Kunz CH. Bestimmung von Schwellenwerten (cut of) bei Enzymimmunoassyas am Beispiel des FSME-ELISA. Klin Lab 1993;39: 877-86.
10. Hauser U, Krahl H, Peters H, Fingerle V, Wilske B. Impact of strain heterogeneity on Lyme disease serology in Europe: Comparison of enzyme-linked immunosorbent assays using different species of *Borrelia burgdorferi* sensu lato. J Clin Micobiol (1998) 36:427-36.
11. Wilske B, Fingerle V, Herzer P, Hofmann A, Lehnert G, Peters H, Pfister HW, Preac-Mursic V, Soutschek E, Weber K. Recombinant immunoblot in the serodiagnosis of Lyme borreliosis. Med Microbiol Immunol 1993;182: 255-70.
12. Hamann-Brand A, Breitner S, Schulze J, Brade V. Laboratory diagnosis of Lyme disease. Biotest Bulletin 1995;5:127-42.
13. Hamann-Brand A, Flondor M, Brade V. Evaluation of a passive hemaglutination assay as screening test and of a recombinant immunoblot as confirmatory test for serological diagnosis of Lyme-disease. Eur J Clin Microbiol Infect Dis 1994;13:572-5.
14. Weber B, Brade V, Doerr HW. Durch Zecken übertragene Erkrankungen. Münch Med Wschr 1991;133:441-6.
15. Brede, HD. FSME - ein Situationsbericht. Notabene Medici 1997; 4:165-6.
16. Anonymus. FSME-Fälle ansteigend - keine Statistik für Borreliose. Der Allgemeinarzt 1996;13:1425-6.
17. Bundesgesundheitsinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (BgVV) und Robert-Koch-Institut (RKI). Frühsommer-Menigoenzephalitis (FSME) - Erkennung und Verhütung. Merkblatt für Ärzte. Bundesgesbl 1996;39:406-8.
18. Verdon ME, Sigal LH. Recognition and management of Lyme disease. Am Fam Physician 1997;56:427-36.
19. Radsel-Medvescek A, Marolt-Gomiscek M, Gajsek-Zima M. Clinical characteristics of patients treated at the University Medical Center Hospital for infectious diseases in Lublijana during the years 1974 and 1977. Zentralbl Bakteriol 1980; Suppl 9: 277-80.
20. Duniewicz M. Klinisches Bild der zentraleuropäischen Zeckenenzephalitis. Münch Med Wschr 1976;118:1609-12.
21. Aebi C, Schaad UB. FSME Immunglobulin- eine kritische Beurteilung der Wirksamkeit. Schweiz Med Wschr 1994;124:1837-40.
22. Kluger G, Schöttler A, Waldvogel K, Nadal, D, Hinrichs W, Wündisch GF, Laub MC. Tick-borne encephalitis despite specific immunoglobulin prophylaxis. Lancet 1995;346:1502.