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MAJOR ARTICLE

Prospective Assessment of the Etiology of Acute Febrile Illness after a Tick Bite in Slovenia

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A prospective study established the etiology of febrile illnesses in residents of Slovenia that occurred within 6 weeks after a tick bite. A combination of laboratory and clinical criteria identified 64 (49.2%) of 130 patients as having confirmed, probable, or possible cases of tickborne disease during 1995 and 1996. Of the 130 patients, 36 (27.7%) had laboratory evidence of tickborne encephalitis, all of whom had clinically confirmed disease. Evidence of infection with *Borrelia burgdorferi* sensu lato was identified in 26 patients; 10 (7.7%) had confirmed Lyme borreliosis. Of 22 patients with evidence of *Ehrlichia phagocytophila* infection, 4 (3.1%) had confirmed ehrlichiosis. Infection by multiple organisms was found in 19 (14.6%) of 130 patients. Patients with meningeal involvement (43 [72.3%] of 59) were more likely to have confirmed tickborne disease than were patients with illness of undefined localization (18 [26.5%] of 68; $P < .0001$). Tickborne viral and bacterial infections are an important cause of febrile illness in Slovenia.

Slovenia, a small central European country with 2 million inhabitants, has been recognized for >50 years as a region where tickborne encephalitis (TBE) is endemic, and since 1986, Lyme borreliosis has also been known to be endemic there. The annual incidence of both diseases is among the highest in Europe [1]. In 1998, we documented the first 4 cases of human granulocytic ehrlichiosis (HGE), caused by *Ehrlichia phagocytophila*, in Slovenia, indicating the presence of yet another endemic tickborne pathogen [2]. *Ixodes ricinus*, the most common tick species in Slovenia, is the main vector of all 3 pathogens [3, 4]. This tick is widely dispersed in Slovenia, a country of 20,256 km^2, of which one-half is covered by forests.

Every summer, numerous patients with febrile illnesses that have occurred after a tick bite are seen by physicians at our medical center. The etiology of these illnesses has never been systematically examined. Herein we summarize the results of a prospective study in which we combined clinical criteria with laboratory diagnostic confirmation to establish the etiology of febrile illnesses occurring in a cohort of patients enrolled during a 2-year period.

PATIENTS AND METHODS

Prospective enrollment. From January 1995 through December 1996, we enrolled patients who presented with possible tickborne infection to the Department of Infectious Diseases, University Medical Centre, Ljubljana, Slovenia. Enrollment criteria included fever (tem-

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Informed consent was obtained from all patients included in the study.

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perature ≥38°C), history of a tick bite (received within the previous 6 weeks), age >15 years, and no obvious other cause of the illness (such as community-acquired pneumonia, urinary tract infection, erysipelas, or sepsis). At first visit, patients were assigned to 1 of 3 clinical groups on the basis of the following criteria: (1) erythema migrans present, (2) possible meningeal involvement, and (3) localization of illness undefined.

Specimen collections. From each patient, an acute-phase serum specimen and EDTA-anticoagulated whole blood specimen were collected. Additional patient evaluations and serum collections were done at 14 days and at 6–8 weeks after the onset of disease. Lumbar puncture was performed if meningeal involvement was suspected.

Clinical laboratory testing. At the first visit, Giemsa-stained peripheral blood smears were examined by light microscopy for the presence of ehrlichial morulae within leukocytes. A complete blood cell count and serum chemistry profile were determined at each evaluation. Cell counts and concentrations of total protein, immunoglobulin, glucose, and albumin in CSF samples were determined.

Culture of* Borrelia burgdorferi *sensu lato. For patients with erythema migrans, a biopsy specimen was obtained from the periphery of the skin lesion, as described elsewhere [5], and it was cultured in modified Kelly-Pettinkofor medium monobasic potassium phosphate medium [6]. CSF specimens were also cultured in modified Kelly-Pettinkofor medium for the presence of borreliae.

Diagnostic testing of serum and CSF samples. The presence of IgM and IgG antibodies to TBE virus was assessed by means of ELISA (Immunozyme, FSME; Immuno AG), performed according to the manufacturer's instructions. Demonstration of the presence of IgM antibodies to TBE virus or seroconversion by IgG ELISA (after negative findings at initial examination) in sequentially obtained serum samples was considered an indicator of a recent infection. The presence of IgG antibodies without IgM, in unchanging titers, was interpreted as evidence of previous TBE.

We used indirect immunofluorescence assays (IFAs) to test for presence of IgM and IgG antibodies to whole cells of a local isolate of *Borrelia afzelii* [7], *E. phagocytophila* antigens [8], *Ehrlichia chaffeensis* antigens (MRL Diagnostics), and *Rickettsia conorii* antigens (bioMérieux). For the purposes of this study, serum antibody titers ≥1:256 for *B. burgdorferi* sensu lato, ≥1:128 for *E. phagocytophila* or *E. chaffeensis*, and ≥1:80 for *R. conorii* were interpreted as positive. Antiborrelial IFA IgM and IgG antibody titers in CSF ≥1:8 were interpreted as positive. Intrathecal borrelial-antibody production was demonstrated by a *B. burgdorferi* IgM and/or IgG index >2 [9].

PCR assays. DNA was extracted from leukocytes separated from blood in the buffy coat and used as a template for PCR assays to detect DNA from the *E. phagocytophila* genogroup and *E. chaffeensis* as described elsewhere [2]. The primers used were 16S rRNA gene primers GE9f and GE10r and nested primer sets (HS1/HS6 followed by HS43/HS45) that target the *GroESL* operon of ehrlichiae [4].

Case definitions. A case of TBE was considered confirmed by the following findings: fever, clinical signs/symptoms of meningitis or meningoencephalitis, an elevated CSF cell count ($> 5 \times 10^6$ cells/L), and serum IgM antibodies to TBE virus and/or IgG seroconversion to TBE virus.

A case of Lyme borreliosis was considered confirmed by the following findings: erythema migrans and/or other typical or highly suggestive clinical signs (e.g., lymphocytoma, Bannwarth's syndrome, or meningitis) and 1 of the following: (1) isolation of *B. burgdorferi* sensu lato from skin or CSF, (2) seroconversion to borrelial antigens, or (3) *B. burgdorferi* sensu lato intrathecal antibody production. Patients who met the clinical criteria but not the strict laboratory-test criteria and had positive borrelial serum antibody titers were considered to have probable Lyme borreliosis. Patients with febrile illness who did not fulfill the clinical requirements but had laboratory evidence of borrelial infection were considered to have possible Lyme borreliosis.

A case of HGE or human monocytic ehrlichiosis (HME) was considered confirmed by the following findings: fever and seroconversion, or a 4-fold change in serum antibody titer to *E. phagocytophila* or *E. chaffeensis* antigens, and/or a positive PCR with subsequent sequencing of the amplicons that demonstrated *Ehrlichia*-specific DNA. A case of acute HGE or HME was defined as probable if the patient had fever and acute and convalescent serum samples with unchanging IFA titers (i.e., ≤2-fold difference) of antibodies to *E. phagocytophila* or *E. chaffeensis* antigens (≥1:128). A case of HGE or HME was considered possible if the patient had serum samples with a titer of ≥1:128 of antibodies to *E. phagocytophila* or *E. chaffeensis* at only 1 testing point.

Data analysis. Differences in quantitative data were analyzed by Kruskal-Wallis tests, and differences in qualitative data by the χ^2 or Fisher's exact test, with use of Epi Info software, version 6.04 (Centers for Disease Control, Atlanta). All *P* values were 2-tailed; those <.05 were considered statistically significant.

RESULTS

Frequency of tickborne disease. During the 2-year study, 130 patients were enrolled and 64 (49.2%) received a diagnosis of ≥1 tickborne disease. The most common disease was TBE, followed by Lyme borreliosis and HGE (table 1). A single diagnosis was made for 45 individuals, whereas 19 patients were potentially infected with >1 tickborne organism. No cases of

Table 1. Confirmed, probable, and possible etiologies of febrile illnesses following a tick bite for 130 individuals in Slovenia.

Diagnosis and case classification	No. (%) of patients	Method of determination[a]
Tickborne encephalitis, confirmed	36 (27.7)	IgM antibody and clinical signs of aseptic meningitis (n = 36)
Lyme borreliosis		
Confirmed	10 (7.7)	Clinically compatible disease and isolation of *B. burgdorferi* (n = 3), seroconversion (n = 7), or development of intrathecal antibodies (n = 7)
Probable	3 (2.3)	Stationary antibody titers and clinically compatible disease
Possible	13 (10.0)	Antibody and fever; no other clinical signs
Human granulocytic ehrlichiosis		
Confirmed	4 (3.1)	PCR demonstration of ehrlichial DNA (n = 3) or seroconversion (n = 4)
Probable	14 (10.8)	Multiple antibody titers ≥1:128
Possible	4 (3.1)	Single antibody titer ≥1:128
Human monocytic ehrlichiosis		
Confirmed[b]	1 (0.8)	Seroconversion
Probable	2 (1.5)	Multiple antibody titers ≥1:128

[a] More than 1 method of determination may have been used to identify a single case.
[b] Diagnosis of HGE was also confirmed.

Mediterranean spotted fever were diagnosed (on the basis of detection of *R. conorii* antibodies).

Cases of TBE. Cases of TBE were confirmed by detection of IgM antibodies in 36 (27.7%) of 130 patients; in all 36, IgG antibodies were also found. Antibodies reactive to ≥1 additional tickborne organism were present in 13 (36.1%) of the 36 patients with TBE (table 2).

All 36 patients with IgM antibodies to TBE virus had lymphocytic meningitis or meningoencephalitis. A typical biphasic course of illness was established for 25 (69.4%) of 36 patients. All 7 patients who were seronegative at initial examination (as determined by IgM ELISA) seroconverted in within 6 weeks after the onset of illness.

An additional 26 (20%) of the 130 enrolled patients had IgG but not IgM antibodies to TBE virus (data not shown). Three of these 26 (11.5%) had been vaccinated against TBE, and 14 (53.8%) had a history of TBE. Nine patients (34.6%) had no history of illness compatible with meningitis, a circumstance that suggests they may have had asymptomatic infections with TBE virus in the past.

Cases of Lyme borreliosis. Overall, 10 (7.7%) of 130 patients with confirmed borreliosis were identified by ≥1 diagnostic methods (table 1). Isolates of *B. burgdorferi* sensu lato were obtained from the skin lesions of each of the 3 febrile patients with erythema migrans. In 7 patients who had CSF samples with detectable borrelial-antibody titers, evidence of intrathecal antibody production was detected (median *B. burgdorferi* IgG index, 7.3; range, 3.2–10.7), and 6 of these 7 patients had lymphocytic meningitis. An additional 7 patients seroconverted to borrelial antigens, including 3 in whom production of borrelial intrathecal antibody was also detected.

In addition to the 10 patients with confirmed cases of Lyme borreliosis, 3 patients with probable cases (2.3% of enrolled patients) were identified. Each patient had meningitis and no positive borrelial serum antibody titers. In addition, 13 patients with possible Lyme borreliosis (10%) were found to have positive borrelial-antibody titers, but without characteristic clinical signs of Lyme borreliosis. Two possible cases involved previously diagnosed Lyme borreliosis, but no clinical information on previous borrelial infection was elicited in the remaining 11 cases.

Several cases in which there was evidence of *B. burgdorferi* sensu lato infection met the criteria for diagnosis of an alternative disease, most often TBE (table 2). Among confirmed cases of Lyme borreliosis, 6 of 10 (60%) also fulfilled the criteria for TBE; all 3 patients with probable Lyme borreliosis had TBE, but none of the 13 patients with possible Lyme borreliosis had TBE (table 2). Overall, antibody to >1 tickborne organism was found significantly more often in patients with confirmed Lyme borreliosis than in patients without obvious clinical signs (7 of 10 vs. 3 of 13; P = .0397).

Cases of HGE. Although no intracytoplasmic morulae in leukocytes were found on any blood smear examinations, IgG antibodies to *E. phagocytophila* at a titer ≥1:128 were found in 22 patients (16.9%). Four patients had confirmed HGE diagnosed on the basis of seroconversion to *E. phagocytophila* antigens (table 1).

For 3 of 4 patients with serologically confirmed cases of HGE, PCR analysis with use of 16S rRNA primers GE9f/GE10r and the *GroESL* primers generated products of the expected sizes (919 bp and 480 bp, respectively) from DNA extracted from blood samples obtained at the first visit. Sequencing of these amplicons confirmed the DNA to be of ehrlichial origin, as reported elsewhere [2].

In 14 (63.3%) of 22 patients, titers of antibody to *E. phagocytophila* were consistently ≥1:128 in repeated tests, meeting the study criteria for probable HGE. In addition, 4 (18.2%) of 22 patients met the criteria for possible HGE. However, 3 had a reliable alternative explanation for their illness; all 3 fulfilled study criteria for TBE and 1 also for neuroborreliosis (table 2).

In 13 (59.1%) of the 22 patients with antibodies to *E. phagocytophila*, serum antibodies to other tickborne organisms were also detected (table 2). Seven (50%) of the patients with probable HGE met the study criteria for a confirmed, probable, or possible case of another tickborne disease: for 4 patients, TBE (2 of these 4 also fulfilled criteria for confirmed Lyme borreliosis); for 2 patients, possible Lyme borreliosis; and for 1, probable acute HME.

Cases of HME. None of the 3 patients with antibodies to *E. chaffeensis* had PCR results that were positive for this organism. Serum antibodies from all 3 of these individuals were dually reactive to *E. chaffeensis* and *E. phagocytophila* antigens, and it is likely that these infections were caused by *E. phagocytophila.*

***Testing for* R. conorii.** Serum antibodies to *R. conorii* were not detected in any of the patients studied.

Clinical and epidemiological features of patients. The 130 patients were assigned to 1 of 3 clinical groups on the basis of the following criteria: erythema migrans present (3 patients [2.3%]), possible meningeal involvement (59 patients [45.4%]), and localization of illness undefined (68 patients [52.3%]). Overall, there was a slight excess of males in the study population (71 patients [54.6%]; table 3).

Erythema migrans. The 3 patients with erythema migrans were women aged 36, 45, and 55 years. The median duration of the incubation period from tick bite to onset of disease was 11 days (range, 10–14 days), and the median duration of fever was 4 days (range, 3–5 days; table 3). *B. burgdorferi* sensu lato was isolated from biopsy specimens of the skin lesion in all 3 cases.

Patients with possible meningeal involvement and illness of undefined localization. Our success in establishing an etiology for disease was significantly higher for patients with signs of meningeal involvement (43 [72.3%] of 59;) than for patients with illness of undefined localization (18 [26.5%] of 68 patients; $P < .0001$). The most frequent diagnosis among patients with clinically suspected meningitis was TBE (table 4). The diagnosis of TBE was limited to this group and, even among them, limited to patients with lymphocytic meningitis. The group with suspected meningitis was also more likely to have diagnostic test results that were concurrently positive for an additional tickborne organism (table 4).

Patients with possible meningeal involvement were younger, had a longer duration of fever, and were more likely to be hospitalized than patients with localization of illness undefined (table 3). The majority of patients with possible meningeal involvement presented from May through October, similar to when patients with illness of undefined localization presented (from April through November). Patients with clinical signs of meningitis were more likely to recall multiple tick bites during the 6 weeks prior to the onset of illness (table 3).

Of the 59 patients with clinical signs of meningeal involvement, CSF examination revealed lymphocytic meningitis in 42 (71.2%). The 42 patients with abnormal CSF findings differed in several ways from the 17 patients for whom there was clinical suspicion of meningitis but whose CSF findings were normal. Patients with abnormal CSF findings were older (median of 45.5 years [range, 17–85 years] vs. median of 27 years [range, 18–58 years]; $P < .001$), had shorter incubation periods (median of 7 days [range, 2–24 days] vs. median of 14 days [range, 5–30 days]; $P = .002$), and tended to have a longer duration of fever (median of 7 days [range, 2–15 days] vs. median of 5 days [range, 2–13 days]). These 2 classes of patients did not differ with regard to sex, number of tick bites, and hospitalization rate.

Table 2. Results of serological testing for multiple tickborne pathogens in 130 patients with an acute febrile illness following a tick bite.

Antigen(s) to organism	No. (%) of seropositive patients[a]
TBE virus only	23 (36.5)
Borrelia burgdorferi sensu lato only	12 (19.1)[b]
Ehrlichia phagocytophila only	9 (14.3)
Ehrlichia chaffeensis only	0
Rickettsia conorii	0
TBE virus, *B. burgdorferi* sensu lato	6 (9.5)
TBE virus, *E. phagocytophila*	4 (6.4)
TBE virus, *E. phagocytophila, B. burgdorferi* sensu lato	3 (4.8)
E. phagocytophila, B. burgdorferi sensu lato	3 (4.8)[c]
E. phagocytophila, E. chaffeensis	2 (3.2)
E. phagocytophila, E. chaffeensis, B. burgdorferi sensu lato	1 (1.6)

NOTE. TBE, tickborne encephalitis.

[a] Of 130 patients who were tested for antibodies to different tickborne pathogens, 63 (48.7%) were seropositive to ≥1 of the tested pathogens.

[b] In addition to 12 patients with positive serology, laboratory evidence of infection was demonstrated in one seronegative patient with erythema migrans by the isolation of *B. burgdorferi* sensu lato from the skin lesion.

[c] One patient with meningitis had only a positive CSF IgG antibody titer to *B. burgdorferi* sensu lato.

DISCUSSION

Tickborne pathogens are a significant cause of morbidity in Europe and North America, where there are tens of thousands

Table 3. Findings for patients with an acute febrile illness following a tick bite.

Variable	Diagnosis[a]			
	Erythema migrans (n = 3)	Possible meningeal involvement (n = 59)	Localization of illness undefined (n = 68)	P[b]
Sex, male/female	3/0	26/33	30/38	.862[c]
Age, years				
Mean ± SD	45.3	44.7 ± 16.9	33.5 ± 14.6	.002[d]
Median (range)	45 (36–55)	41 (16–85)	33 (16–70)	
Incubation period, days				
Mean ± SD	11.7	11.3 ± 5.6	11.9 ± 7.0	.970[d]
Median (range)	11 (10–14)	10 (2–30)	11 (2–30)	
Duration of fever, days				
Mean ± SD	4.0	6.8 ± 3.0	5.3 ± 2.9	.007[d]
Median (range)	4 (3–5)	7 (2–15)	5 (2–21)	
Patient characteristics, no. (%) of patients				
Hospitalized	2 (67)	54 (92)	3 (5)	.000[c]
>1 Tick bite[e]	0	29 (49.2)	16 (23.5)	.004[c]

[a] For case definitions see Methods.
[b] Comparison of the group of patients with possible meningeal involvement and the group with localization of illness undefined.
[c] χ^2 test (2-tailed).
[d] Kruskal-Wallis test.
[e] During the 6 weeks prior to onset of the present illness.

of cases of Lyme borreliosis annually [10]. Other tickborne pathogens, including viruses (e.g., TBE virus) and intracellular bacteria (e.g., *Rickettsia rickettsii, E. chaffeensis,* and *E. phagocytophila*), can cause fatal infections in humans [11, 12]. Few studies have attempted to identify the varied etiologies of febrile illnesses occurring in persons with a history of a recent tick bite.

Most studies exploring the disease burden posed by tickborne pathogens have relied on indirect measures of tick exposure rather than a history of a recent tick bite. These studies involve selecting cohorts of persons exposed to tick-infested habitats [13, 14], choosing study populations from locations where tickborne diseases are endemic [15, 16], or testing samples obtained from patients hospitalized during the summer months with fevers of unknown origin [17]. Relatively few studies have looked at cohorts or case series of ill patients with a history of recent tick bite, although a number of studies have prospectively followed patients who received tick bites, to assess the risk of becoming ill [18, 19] and to assess the effectiveness of prophylactic antibiotic treatment [20, 21]. Examining febrile patients with a known history of tick bite has been instrumental in identifying diseases caused by newly described pathogens, such as HME caused by *E. chaffeensis* [22, 23].

In our study we attempted to determine the etiology of acute febrile illness in adults who had been bitten by a tick in a central European country where several tickborne diseases are endemic. We examined patients for laboratory evidence of HME and Mediterranean spotted fever, in addition to Lyme borreliosis, TBE, and HGE. We were able to ascribe an etiology for the illness in >49% of the enrolled patients.

It was not surprising that for more than one-half of the patients with a history of tick bite in the 6 weeks before onset of illness, a specific tickborne illness could not be diagnosed. There are many febrile illnesses that occur during "tick season" that are unrelated to the occurrence of a coincidental tick bite. In addition, we did not test for diseases caused by other tickborne organisms (e.g., babesiosis). Since novel tickborne pathogens of humans continue to be described (e.g., *Ehrlichia ewingii*) [24], it is possible that some cases of tickborne disease might have been missed (although *E. ewingii* is not known to occur in Europe).

Among patients with laboratory evidence of a disease caused by a tickborne organism, the most common infections diagnosed were due to TBE virus (36 of 130 patients), *B. burgdorferi* sensu lato (26 of 130), and *E. phagocytophila* (22 of 130). A number of the patients had evidence of >1 infection, although in most cases a more likely etiology could be identified. Serological evidence of infection with *E. chaffeensis* was found in 3 patients. However, we believe these individuals were infected by *E. phagocytophila* and that HGE was the only ehrlichial

Table 4. Diagnosis according to case definitions of study for patients with an acute febrile illness following a tick bite.

Diagnosis[a]	No. (%) of patients with diagnosis			
	Erythema migrans (n = 3)	Possible meningeal involvement (n = 59)	Localization of illness undefined (n = 68)	P[b]
TBE (all)	0	36/59 (61.0)	0/68	0
TBE only	0	23/36 (63.9)	0	
TBE and ⩾1 other	0	13/36 (36.1)	0	
LB (all)	3/3	13/59 (22.0)	10/68 (17.2)	.402
Confirmed	3/3	7/13 (53.8)	0	.008
Probable	0	3/13 (23.1)	0	.229
Possible	0	3/13 (23.1)	10/10 (100)	.000
LB only	3/3	3/13 (23.1)	7/10 (70)	.040
LB and ⩾1 other	0	10/13 (76.9)	3/10 (30)	.040
HGE (all)	0	11/59 (18.6)	11/68 (16.2)	.895
Confirmed	0	1/11 (9.1)	3/11 (27.3)	.587
Probable	0	6/11 (54.5)	8/11 (72.7)	.659
Possible	0	4/11 (36.4)	0	.090
HGE only	0	3/11 (27.3)	6/11 (81.8)	.387
HGE and ⩾1 other	0	8/11 (72.7)	5/11 (18.2)	.387
HME (all)	0	0	3/68 (4.4)	—
Confirmed	0	0	1/3 (33.3)	—
Probable	0	0	2/3 (66.7)	—
HME only	0	0	0	—
HME and ⩾1 other	0	0	3/3 (100)	—
Etiology, no. of organisms				
0	0	16/59 (27.1)	50/68 (73.5)	.000
1	3	29/59 (49.2)	13/68 (19.1)	.001
>1	0	14/59 (23.7)	5/68 (7.6)	.020

NOTE. HGE, human granulocytic ehrlichiosis; HME, human monocytic ehrlichiosis; LB, Lyme borreliosis; TBE, tickborne encephalitis.

[a] According to the case definitions used in this study; see Methods.

[b] Comparison of the group of patients with possible meningeal involvement and the group of patients with localization of illness undefined, as determined by χ^2 or Fisher's exact test (2-tailed).

disease that occurred in our patient cohort. In the United States, some individuals' serum samples are reactive to both *E. chaffeensis* and *E. phagocytophila* antigens [25], which indicates the potential for cross-reactive antibodies to develop.

In many of the cases in which laboratory findings indicated tickborne infection, our strict case-definition criteria for confirmed disease were met unambiguously. All the patients with a febrile illness and IgM antibody to TBE virus had lymphocytic meningitis. However, only 10 of 26 patients with laboratory evidence of infection with *B. burgdorferi* sensu lato met the study criteria for confirmed Lyme borreliosis; similarly, that diagnosis could be confirmed for only 4 of 22 patients with laboratory evidence of HGE.

We must stress that our case definitions were intentionally strict and presumably resulted in some classification of cases as probable or possible when patients presented with generalized complaints. For example, a confirmed diagnosis of Lyme borreliosis required the presence of typical clinical signs of the disease. Thus, patients without typical disease were classified as having possible Lyme borreliosis, even if laboratory analyses indicated either recent seroconversion or evidence of specific intrathecal antibody production. Asymptomatic Lyme borreliosis is relatively frequent; therefore, seroconversion alone is not proof of the etiology of the current febrile disease [26]. Furthermore, intrathecal antibody production is a well-recognized and important laboratory indicator of neuroborreliosis, but it may persist with normal CSF cell counts for several additional months or even years after acute infection [27].

An additional finding of interest in this study was the high level of antibodies to tickborne organisms, which indicated remote rather than acute infection. As an example, the number of patients with solely IgG antibody to TBE virus in our study

was high (26 [20%] of 130 patients) yet within the range expected for regions where this disease is endemic [28]. However, even with an average incidence of TBE of 20 cases per 100,000 residents in central Slovenia [29], it is still striking that 14 of 130 patients had a history of TBE. Similarly, 16 of 130 patients (12.3%) had possible or probable Lyme borreliosis. A background seroprevalence of 5.3% has been described with regard to an adult population in rural central Slovenia [30]. No published data exist on the background prevalence of antibodies reactive to *Ehrlichia* antigens in adult Slovenian populations.

The currently studied patient population in central Slovenia was highly exposed to ticks and at high risk for acquiring multiple tickborne pathogens. A relatively large proportion of patients had laboratory indications of infection with >1 tickborne organism, and several patients fulfilled the diagnostic criteria for >1 disease. In Slovenia, TBE virus, *E. phagocytophila,* and *B. burgdorferi* sensu lato can be transmitted by *I. ricinus,* thus increasing the potential risk for multiple infection. In fact, concomitant infections with TBE virus and *B. burgdorferi* sensu lato have been described in patients with acute meningitis [31].

Our study revealed that HGE is also present in this area. In the United States, it has been experimentally demonstrated that dually infected ticks can simultaneously transmit *B. burgdorferi* and *E. phagocytophila* [32], and serological evidence of antibodies to multiple tickborne organisms is not uncommon [16, 33]. Unfortunately, the small number of patients in our 3 subgroups did not permit extensive comparisons of clinical and laboratory characteristics between individuals who had a single infection and those who met the definition for multiple infection. The potential clinical relevance of combined infections remains to be defined.

It should also be noted that by restricting our study to adult febrile patients with a known history of tick bite, we may have missed many cases of tickborne disease presenting at our medical center. Our study was not designed to assess the burden of tickborne disease in children <15 years of age. In addition, the percentage of persons recalling a tick bite prior to developing a tickborne illness varies, but reported values are 68% for TBE in Germany [34] and 73% for erythema migrans in Slovenia [35]. In the United States it is estimated that ~73% of patients with HGE [36] and 68% of patients with HME [37] recall receiving a tick bite prior to their illness.

Our ability to identify etiologic agents was greater for patients with symptoms and signs of CNS involvement or erythema migrans than for patients with illness of undefined localization. The most important reason for this difference was that TBE virus caused disease solely in the group of patients who had meningitis.

Definitively documenting the etiology of meningitis for patients with borrelial infection was more difficult. The essential criterion for proven neuroborreliosis among patients with meningitis is the intrathecal production of borrelial antibodies. To prove local antibody synthesis, it is necessary to show that CSF antibody titers are higher than they would be expected to be due to leakage from serum into CSF. Although various formulas have been applied for the assessment of intrathecal antibody production, it is generally agreed that the CSF:serum antibody index should be >2 [9, 38]. Unfortunately, calculating such indices on the basis of IFA titers of 2-fold serial dilutions is an approximate method, because inexact readings can amplify inaccuracies. Typically, IFA titers within a single dilution (i.e., a 2-fold difference) are not considered significantly different, as this range of variation is inherent to the test. However, such variation can dramatically effect indices based on endpoint–titer ratios. To be significant, index values should probably be ⩾4. Elevated indices in our patients range from 3.2 to 10.7. In 6 of 7 patients the index was >4; in 3 of the 7 patients, including the patient with an index of 3.2, seroconversion to borrelial antigens was also established.

With the combined clinical and laboratory methods used in our study, tick-transmitted diseases were diagnosed for nearly half of the patients with an acute febrile illness occurring within 6 weeks following a tick bite. Many of these patients met strict case-definition criteria for each disease. Relatively often, evidence of concurrent or past infection with >1 tickborne organism was found. Investigations that include younger patients are warranted to more clearly define the public health importance of disease caused by tickborne pathogens in Slovenia.

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