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Causes of febrile illnesses after a tick bite in Slovenian children

MAJA ARNEŽ, MD, PHD, TATJANA LUŽNIK-BUFON, MD, TATJANA AVŠIC-ŽUPANC, PHD, EVA RUŽIC-SABLJIC, MD, PHD, MIROSLAV PETROVEC, MD, PHD, STANKA LOTRIČ-FURLAN, MD, PHD AND FRANC STRLE, MD, PHD

Background. **To establish the etiology in Slovenian children with febrile illnesses occurring after a tick bite.**

Methods. **Eighty-six febrile patients younger than 15 years referred to our institution in 2001 with a history of a tick bite within 6 weeks before onset of the illness were included in this prospective study. Acute and convalescent serum samples were tested for the presence of antibodies to tick-borne encephalitis virus, *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum, Ehrlichia chaffeensis, Rickettsia conorii, Babesia microti, Bartonella henselae, Bartonella quintana* and *Francisella tularensis.* Cerebrospinal fluid was investigated in patients in whom meningeal involvement was clinically suspected. Blood and/or cerebrospinal fluid from the patients were cultured in modified Kelly-Pettenkofer medium. PCR was performed to detect ribosomal DNA of *A. phagocytophilum* and *E. chaffeensis.***

Results. **Of 86 patients 33 (38%) were excluded because a well-defined febrile illness not associated with tick bite was established. Tick-borne illness was diagnosed in 28 (53%) of the 53 remaining patients. The most common diagnosis was tick-borne encephalitis (64%), followed by Lyme borreliosis (46%), human monocytic ehrlichiosis and human granulocytic ehrlichiosis (serologic evidence of infection in 9 and 4%, respectively). In 6 (21%) patients there was evidence for infection with more than 1 tick-borne agent.**

Conclusions. **Tick-borne illness was established in 53% of the patients younger than 15 years presenting with febrile illness occurring within 6 weeks after a tick bite. The most common identified illnesses were tick-borne encephalitis and Lyme borreliosis.**

INTRODUCTION

During recent decades several newly recognized and potentially fatal tick-borne illnesses have been reported in humans.[1, 2] At least three of them are endemic in Slovenia: tick-borne encephalitis (TBE) caused by TBE virus; Lyme borreliosis (LB) caused by *Borrelia burgdorferi* sensu lato (the term represents the strain that has not been identified to the species level); and human granulocytic ehrlichiosis (HGE) caused by the Gram-negative intracellular bacterium *Anaplasma phagocytophilum*.[3] The main vector of all three pathogens in this area is the tick *Ixodes ricinus*.[4, 5]

The stimulus for the present study was the report on

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From the Department of Infectious Diseases, University Medical Centre Ljubljana (MA, SLF, FS), and the Medical Faculty, Institute of Microbiology and Immunology, University of Ljubljana (TLB, TAŽ, ERS), Ljubljana, Slovenia.
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Address for reprints: Maja Arnež, M.D., Ph.D., Department of Infectious Diseases, University Medical Centre, Japljeva 2, 1525 Ljubljana, Slovenia. Fax 3861 522 2456; E-mail maja.arnez@kclj.si.

the etiology of acute febrile illnesses after a tick bite in Slovenian adult patients[3] and the documentation of the first confirmed European pediatric case of HGE.[6] This study was performed in order to establish the etiology of febrile illnesses occurring after a tick bite in Slovenian children.

MATERIALS AND METHODS

Patients and study design. The project was approved by the Medical Ethics Committee at the Ministry of Health of the Republic of Slovenia. Informed consent for participation in the study was obtained from parents or guardians of all patients. Febrile patients (≥38°C) younger than 15 years, referred to our institution between March 1 and December 31, 2001, with a history of a tick bite within 6 weeks before onset of their illness, qualified for this prospective clinical study.

All patients represents spontaneous referrals from physicians of Ljubljana region (central part of Slovenia).

Methods. Medical history, physical examination, basic hematologic, biochemical and microbiologic investigations were performed at the time of the first visit as well as 14 days and 6 weeks later. The methodologic approach was similar to that described previously for adult patients.[3] For each patient a serum specimen and EDTA-anticoagulated whole blood sample were collected at each visit. Erythrocyte sedimentation rate, C-reactive protein concentration, complete blood count and serum levels of aspartate aminotransferase, alanine aminotransferase, creatinine phosphokinase, urea, creatinine and blood glucose were determined. Lumbar puncture with cerebrospinal fluid (CSF) investigation (cell counts, concentrations of protein, albumin, immunoglobulins and glucose) was also performed if meningeal involvement was clinically suspected. A CSF white blood cell count $\geq 5 \times 10^6$/l was considered abnormal and suggestive of meningitis.

Patients were excluded from the study if a well-established (etiologic) diagnosis of a febrile illness not associated with tick bite was documented such as urinary tract infection, pneumonia, diarrhea, streptococcal pharyngitis, etc.

Diagnostic testing of serum and CSF samples. *Culture of* B. burgdorferi *sensu lato.* One milliliter of blood and/or CSF was obtained from patients and placed directly in the modified Kelly-Pettenkofer medium for culture.[7] Culturing was performed as reported previously.[8–10]

PCR. DNA was extracted from leukocytes separated from blood in the buffy coat and used as a template for PCR assays to detect DNA of *A. phagocytophilum* genogroup and *Ehrlichia chaffeensis*. The primers used were 16S ribosomal RNA gene primers Ehr521 and Ehr790 and the second set of nested primers (HS1/HS6 followed by HS43/HSVR) that target the GroESL operon of ehrlichiae.[11, 12]

Antibody determination. Borrelial immunofluorescent assay (IFA) of IgM and IgG antibody titers without preabsorption were determined in serum and CSF.[9, 10] *Borrelia afzelii*, the most frequently isolated strain from humans in Slovenia, was used as an antigen. Titers of ≥256 in serum and ≥16 in CSF were considered positive. The presence of intrathecal antibody production was assessed by commonly used formulas.[13]

The presence of IgM and IgG antibodies to TBE virus was determined by means of ELISA (DADE-Behring; Anti-FSME-virus IgG/IgM) according to the manufacturer's instructions. The presence of IgM antibodies or the appearance of IgG antibodies after negative findings at initial examination (seroconversion) was considered an indicator of a recent infection. The presence of IgG in unchanging titers without IgM was interpreted as evidence of previous TBE virus infection.[3]

Antibodies reactive to *A. phagocytophilum*, *E. chaffeensis*, *Babesia microti*, *Bartonella henselae*, *Bartonella quintana* (Focus Technologies) and *Rickettsia conorii* (Aventis Pasteur) antigens were assessed with IFA. Serum antibody titers of ≥256 were interpreted as positive.[3, 14]

Specific IgM and IgG antibodies to *Francisella tularensis* were determined with ELISA previously described by Carlsson et al.[15]

Case definitions. LB was considered confirmed by the presence of: (1) erythema migrans diagnosed according to slightly modified CDC criteria[16, 17]; (2) other typical or highly suggestive clinical sign of LB (such as meningitis, meningoradiculitis, cranial nerve involvement, ear lobe lymphocytoma) associated with strict laboratory test criteria including isolation of *B. burgdorferi* sensu lato from blood and/or CSF and/or seroconversion to borrelial antigens and/or *B. burgdorferi* sensu lato intrathecal antibody production; or (3) febrile illness without typical or highly suggestive clinical signs of LB but with the isolation of *B. burgdorferi* sensu lato from blood and/or CSF.

Patients without EM who met the clinical criteria but not strict laboratory test criteria and had detectable serum antibodies to *B. burgdorferi* sensu lato were considered to have probable LB.

Patients with febrile illness who did not fulfil the clinical criteria but had laboratory evidence of borrelial infection other than isolation of *B. burgdorferi* sensu lato from blood and/or CSF, were considered to have possible LB.

TBE was considered confirmed in patients with CSF pleocytosis associated with serum IgM antibodies to TBE virus or IgG seroconversion to TBE virus. Febrile patients who demonstrated identical serologic criteria

but had normal CSF findings were considered to have the initial phase of TBE.

HGE or human monocytic ehrlichiosis (HME) was considered confirmed by the following: fever and either seroconversion or at least 4-fold change in serum antibody titer to *A. phagocytophilum* or *E. chaffeensis* antigens 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 positive but unchanging serum IFA titers (i.e. ≤2-fold difference) to *A. phagocytophilum* or *E. chaffeensis*.[3]

A case of acute babesiosis, tularemia, Mediterranean spotted fever and bartonellosis was confirmed in febrile patient with positive IgM antibodies and/or IgG seroconversion or at least 4-fold change in serum antibody titers to *Babesia microti*, *F. tularensis*, *R. conorii* and *B. henselae* or *B. quintana* antigens, respectively.

Statistical analysis. Differences in categoric data were analyzed by the Yates corrected chi square test or Fisher's exact test, whereas differences in continuous data were assessed by Wilcoxon rank sum test. All *P* values were two tailed; $P < 0.05$ was considered statistically significant.

RESULTS

During the 9-month period 86 patients (30 girls, 56 boys) fulfilled the inclusion criteria. The month of the first visit to our department is shown in Figure 1.

Thirty-three (38%) patients were excluded from the study because a well-established (etiologic) diagnosis of a febrile illness not associated with tick bite was documented such as urinary tract infection, pneumonia, diarrhea, streptococcal pharyngitis, etc.

Tick-associated illness was considered possible, probable or confirmed in 28 (53%) of the 53 remaining patients, whereas in 25 of 53 (47%) patients the etiology of the fever after a tick bite was undetermined.

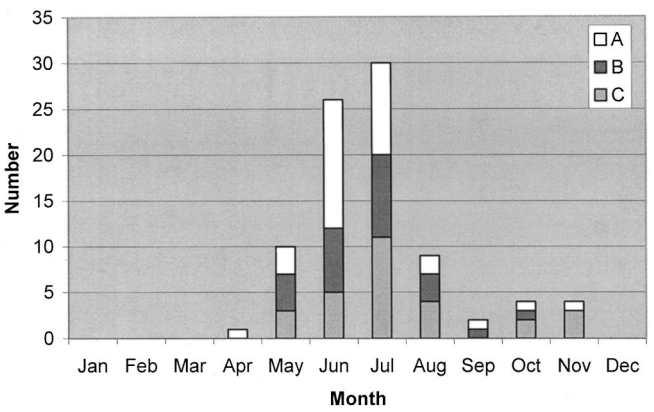

FIG. 1. The month of the first visit in patients with fever after a tick bite. *A,* fever caused by a non-tick-borne illness; *B,* undefined febrile illness associated with a tick bite; *C,* established tick-borne illness.

Demographic features, clinical characteristics and laboratory findings of patients in the two groups are shown in Table 1. Patients with documented tick-borne illness were older, had higher fever and more often a biphasic course of the illness (reappearance of symptoms and signs including fever after improvement or an asymptomatic period of the duration of up to several days) than patients with undetermined disease (Table 1). Fifteen children (10 of 28 with tick-associated illness and 5 of 25 with undetermined etiology; $P = 0.3359$) received empiric treatment with antibiotics such as penicillin, amoxicillin or azithromycin before the first visit at our department; none of them was given doxycycline.

The etiology of established tick-borne illnesses is shown in Table 2. A single diagnosis was made in 22 of 28 (79%) patients, and 6 of 28 (21%) patients showed evidence of infection with more than one tick-borne agent (Table 3).

The most common diagnosis was TBE, representing more than one-half of the illnesses caused by tick-borne agent (Table 2). In all 18 patients with TBE the diagnosis was established by the demonstration of IgM antibodies to TBE virus; in 15 of these patients IgG antibodies were also present at the initial examination whereas in 3 of these patients IgG seroconversion was detected. A typical biphasic course of the disease was found in 12 of 18 (67%) patients; 3 of them were examined in both phases of the illness. One patient with acute fever after a tick bite had IgG but not IgM antibodies to TBE virus (not shown on Table 2); the finding was interpreted as infection with TBE virus in the past (most probably asymptomatic).

Of 13 patients with LB, the diagnosis was confirmed in 11 (84%), probable in 1 (8%) and possible in 1 (8%) patient. Borrelial serum IgG antibodies were detected in only 1 of 7 patients with confirmed LB and meningitis (Table 2); in none of them was intrathecal borrelial antibody production ascertained. In fact we were not able to demonstrate intrathecal production of borrelial antibodies in any of 32 patients in whom specific IgM and IgG antibodies to *B. burgdorferi* sensu lato in CSF were determined. In the present study the isolation rate of *B. burgdorferi* sensu lato from blood was 15% (2 of 13), whereas the isolation rate from CSF was 30% (4 of 13) (Table 2).

Among 53 patients included in our study we could not confirm either evidence of acute or past infection with *Babesia microti*, *F. tularensis* and *R. conorii*. However, IgG antibodies to *B. henselae* were detected in 5 and IgG antibodies to *B. quintana* in 4 patients with fever after a tick bite. In 1 patient with confirmed LB, seroconversion of IgG antibodies to *B. henselae* as well as *B. quintana* was detected.

TABLE 1. Demographic features, clinical characteristics and laboratory findings in 53 patients with febrile illness occurring after a tick bite: comparison of patients with established tick-borne infection and those with undetermined cause of the illness

Variable	Tick-borne Illness	Undetermined Cause of Illness	*P*
No. of patients	28	25	
Female/male	10/18	8/17	0.9956
Age (yr)*	8 (1–14.5)	4.5 (0.5–14)	0.0026
Hospitalization†	27 (96)	20 (80)	0.0889
Incubation (days)‡	15 (1–42)	20 (0–30)	0.5588
Biphasic course†§	12 (43)	3 (12)	0.0290
Duration of illness (days)*	6 (2–30)	4 (1–11)	0.0589
Body temperature (°C)			
Highest*¶	39.6 (38–40.2)	39 (38–40)	0.0230
≥39°C†	23 (82)	13 (52)	0.0402
Leukopenia† only (leukocytes $<4 \times 10^9$/l)	2 (7)	0	0.4987
Thrombocytopenia† only (platelets $<140 \times 10^9$/l)	0	0	
Leukopenia and thrombocyto-penia†	3 (11)	2 (8)	1.0000
Elevated AST and/or ALT†	5 (18)	4 (16)	1.0000
Elevated CK†	3 (11)	3 (12)	1.0000

* Median (range).
† Number of patients (%).
‡ Days from tick bite to the onset of fever.
§ Reappearance of symptoms and signs including fever after improvement or asymptomatic period with the duration of up to several days.
¶ Highest body temperature registered during illness.
AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK, creatinine phosphokinase.

TABLE 2. Etiology of illness in 28 patients with fever after a tick bite and established infection with tick-borne agents

Diagnosis and Case Classification	No.* of Patients	Method of Determination
Tick-borne encephalitis	18 (64)†	
Confirmed	18	Positive serum IgM to TBE virus (18)‡ Seroconversion§ of IgG to TBE virus (3)
Lyme borreliosis	13 (46)	
Confirmed	11	
Solitary EM only	2	Clinical diagnosis (1) Clinical diagnosis and positive borrelial serum antibodies (1)
Solitary EM and meningitis	2	Clinical diagnosis (1) Clinical diagnosis and isolation of *Borrelia burgdorferi* sensu lato from CSF (1)
Multiple EM only	1	Clinical diagnosis
Multiple EM and meningitis	1	Clinical diagnosis
Meningitis and/or meningoencephalitis only	4	Isolation of *B. burgdorferi* sensu lato from blood (2) or CSF (2)
Febrile patient with suspected meningitis	1	Isolation of *B. burgdorferi* sensu lato from otherwise normal CSF
Probable	1	Meningitis and positive borrelial serum an-tibodies
Possible	1	Fever and seroconversion to borrelial anti-gen
Human granulocytic ehrlichiosis	1 (4)	
Probable	1	Fever and antibodies to *Anaplasma phagocytophilum*
Human monocytic ehrlichiosis	3 (9)	
Probable	3	Fever and antibodies to *Ehrlichia chaffeensis*

* Six patients were diagnosed with more than one tick-borne infection.
† Numbers in parentheses, percent.
‡ Numbers in parentheses, number of patients.
§ Fifteen patients had IgG antibodies (in addition to IgM) present at the initial visit.

TABLE 3. Patients with fever after a tick bite with more than one documented tick-borne illness

No. of Patients	Tick-borne Illness	Method of Determination
2	Confirmed TBE + confirmed LB	Meningitis + positive serum IgM TBE + Isolation of *Borrelia burgdorferi* sensu lato from blood
1	Confirmed TBE + probable LB	Meningitis + positive serum IgM TBE + positive serum antibodies to borrelial antigen
1	Confirmed TBE + possible LB	Fever + positive serum IgM TBE + seroconversion to borrelial antigen
1	Confirmed TBE + probable HME	Meningitis + positive serum IgM TBE + positive serum antibodies to *E. chaffeensis* antigen
1	Confirmed LB + probable HGE + probable HME	Meningitis + isolation of *B. burgdorferi* sensu lato from CSF + positive serum antibodies to *Anaplasma phagocytophilum* + Positive serum antibodies to *Ehrlichia chaffeensis*

DISCUSSION

Slovenia, a small central European country, is an endemic region for TBE, LB and HGE.[3, 18] Recently protozoal parasites from *Babesia* spp. were found in Slovenian ticks,[19] but human illness caused by these pathogens has not yet been documented.

The present study, in which combined clinical and laboratory methods were used for the detection of

infection with several tick-borne agents and some agents with potential transmission by tick bites, was performed to establish the causes of febrile illnesses occurring after a tick bite in children. Literature search did not reveal any similar report on pediatric populations but there have been published studies for adult populations.[3, 20–22] Recently the results of a prospective clinical study on the etiology of acute febrile illnesses occurring after a tick bite were reported for a group of adult patients referred to our institution.[3] The present study was performed to obtain information on the etiology and frequency of the tick-borne illnesses in Slovenian children and, because of similar design of the present study and those utilized for adults, to compare the findings in the two age groups.

The findings of the two studies were similar in several ways, including the frequency of diagnosed tick-borne illnesses, which were found in about one-half of patients who developed fever after a tick bite [28 of 53 (53%) in children *vs.* 64 of 130 (49%) in adult patients], as well as the ratio of patients with more than 1 tick-borne illness. Among patients with a disease caused by a tick-borne organism, the most common diagnosis in both studies was TBE, followed by LB. Although the ratios of patients with TBE and LB were comparable, the proportion of patients with HGE was lower in children than in adults [1 of 53 (2%) *vs.* 22 of 130 (17%)]. This result is similar to studies performed in other endemic regions that indicate that HGE and HME in children are rare.[23, 24] We documented the first confirmed European pediatric case of HGE.[6] This finding was one of the reasons for this larger study; however, among 53 children with fever after a tick bite, we found only 1 case with probable HGE. According to the laboratory criteria, we also ascertained 3 patients with probable HME. We would like to stress that none of them fulfilled criteria for confirmed case and that for 2 patients satisfying criteria for probable HME a well-established diagnosis of an alternative tick-borne illness was available.

In this study infection with TBE virus as indicated by the presence of IgM antibodies was established in 18 of 53 (34%) cases. All 18 patients fulfilled criteria for confirmed TBE. The typical biphasic course of the disease was detected in two-thirds of patients, which is equivalent to the proportion found for adult patients.[3] It has been well-recognized that at least in Central Europe the clinical presentation of TBE and the course of the disease somehow differ between adults and children.[25] The disease is as a rule milder and sequelae are less frequent in children than in adults; however, severe forms of TBE in children have also been reported.[26, 27] IgG antibodies (without the presence of IgM) directed to TBE virus indicating past rather than acute infection was found in 2% of children in this study, which is in contrast to 20% as found in adult population.[3] This finding is not unexpected and could be explained by the fact that the seropositivity rate increases with age.[28]

LB was diagnosed in 13 of 53 (25%) patients: 11 fulfilled criteria for confirmed, one for probable and one for possible LB. In comparison to the findings in adults,[3] the proportion of confirmed cases in the present study was higher (11 of 13 *vs.* 10 of 26); whereas the ratio of patients with the diagnosis of possible LB was lower (1 of 13 *vs.* 13 of 26). A reasonable explanation for the lower proportion of possible cases in children is the lower seroprevalence of borrelial antibodies in younger age groups.[28] Of 11 children with confirmed LB, 7 had meningitis. In none of them was intrathecal borrelial antibody production established; in fact we were not able to demonstrate intrathecal production of borrelial antibodies in 32 patients in whom specific IgM and IgG antibodies to *B. burgdorferi* sensu lato in CSF were determined. The probable reason for the failure of this classical diagnostic approach for the demonstration of neuroborreliosis is the examination of CSF very early (during the first few days) in the course of the illness. Contrary to our findings 7 of 10 adult patients with confirmed LB had evidence of intrathecal antibody production to *B. burgdorferi* sensu lato,[3] but the duration of the illness before CSF examination for these patients was longer. *B. burgdorferi* sensu lato was isolated from CSF in 4 of 32 (12%) children who developed febrile illness after a tick bite and had CSF examination performed; excluding patients with TBE the yield would be 4 of 14 (29%). This isolation rate is much higher than found previously in our children with multiple EM (2 of 83, 2%; $P = 0.0036$).[10]

In most of our patients acute and convalescent serum samples were tested for the presence of antibodies to *Babesia microti* and *R. conorii*, which are known tick-borne agents, and to *F. tularensis*, which could also be transmitted by a tick bite. According to the clinical and laboratory criteria we did not find any patient with acute or past babesiosis, tularemia or ritckettsiosis, indicating that these three illnesses are absent or not frequently encountered in Slovenian children experiencing febrile illness after a tick bite. However, past infection with *B. henselae* and *B. quintana*, the agents that have also been found in ticks,[29] was detected in five and four patients, respectively, and in one patient seroconversion of IgG antibodies to both antigens was detected. The finding of seroconversion to the *Bartonella* antigen in a patient with febrile illness occurring after a tick bite might indicate the transmission of the agent through a tick bite but cannot exclude other possible modes of transmission.

In our study the older age of children and the biphasic course of the disease were associated with whereas higher body temperature was suggestive for a

tick-borne illness (Table 1). Contrary to observations in adults,[3] in our patients with fever after a tick bite leukopenia, thrombocytopenia and elevated serum level of liver and muscle enzymes were rare and of no help in distingruishing between the group of patients with established tick-borne illness and those with undetermined cause (Table 1). A reliable explanation for the difference is in distinct etiology with fewer patients with HGE and the initial phase of TBE in the present study.

With a combined clinical and laboratory approach as used in our prospective study, the diagnosis of tick-transmitted illness was established in about one-half of children with febrile illness occurring after a tick bite; most commonly TBE and LB were identified, whereas HGE and HME were rare and not conclusively ascertained. The findings might have impact on the management of febrile children after a known tick bite in central Europe.

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