MAJOR ARTICLE

Tickborne Infections as a Cause of Nonspecific Febrile Illness in Wisconsin

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Lyme disease, human granulocytic ehrlichiosis (HGE), and babesiosis are tickborne infections that are indigenous to Wisconsin. To assess their importance as a cause of nonspecific fever, we recruited patients with febrile illness at 10 clinics in northwestern Wisconsin from May through August of both 1997 and 1998. Eligible patients had a temperature >38.0°C but no rash or other localizing source. Acute and convalescent serological tests were performed for *Borrelia burgdorferi, Babesia microti,* and *Ehrlichia equi*; polymerase chain reaction was performed to detect granulocytic *Ehrlichia* rDNA. Seventeen (27%) of 62 eligible patients had laboratory evidence of tickborne infection, including 7 (11%) with probable Lyme disease only, 8 (13%) with HGE only, and 2 (3%) with apparent coinfection. No patients with *Babesia* infection were identified. Patients with and without tickborne infection were similar with regard to age, sex, symptoms, history of tick bite, and outdoor exposure. The results suggest that tickborne infections are an important cause of nonspecific febrile illness during the tick season in northwestern Wisconsin.

Tick bites are an important source of vector-borne infections in the United States. Pathogens transmitted by ticks include bacteria, viruses, and protozoa [1]. Lyme disease is the most common tickborne infection in the United States, and the incidence of reported cases in Wisconsin ranks eighth among all states [2]. The initial presentation typically includes an expanding erythematous rash (erythema migrans), often accompanied by fever and nonspecific constitutional symptoms. However, Lyme disease may present as constitutional symptoms only, without apparent rash, in 10%–20% of infected individuals [3–5].

Babesiosis is another tickborne infection that has been recognized in Wisconsin since the 1980s [6, 7]. The etiologic agent is *Babesia microti,* a protozoal parasite that invades erythrocytes and causes a malaria-like illness [8]. Patients with babesiosis often require hospitalization, and persistent infection can occur in the absence of specific therapy [9, 10]. Mild or subclinical infection has been documented in the eastern United States, especially in children [11, 12]. Clinically recognized cases of babesiosis appear to be rare in Wisconsin, although statewide surveillance data are not available [13]. Other *Babesia*-like organisms have been reported to cause human illness in other regions of the United States, but not in Wisconsin [14, 15].

The most recently recognized tickborne infection in the upper Midwest is human granulocytic ehrlichiosis (HGE). This disease was first reported in 1994, when a novel *Ehrlichia* agent was identified in a series of patients with severe febrile illness [16]. Analysis of 16S rDNA indicated that the HGE agent is closely related to the veterinary pathogens *Ehrlichia equi* and *Ehrlichia*

Received 5 July 2000; revised 2 October 2000; electronically published 17 April 2001.

The study protocol was approved by an institutional review board, and written informed consent was obtained from all participants.

Financial support: Centers for Disease Control and Prevention (cooperative agreement UR8/CCU513366-01).

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Clinical Infectious Diseases 2001; 32:1434–9

1058-4838/2001/3210-0008$03.00

phagocytophila [17]. The clinical manifestations include a nonspecific febrile illness, which is frequently accompanied by thrombocytopenia, leukopenia, and mild elevations of hepatic enzymes [18, 19]. Approximately 30%–50% of infected patients require hospitalization. The rate of incidence of HGE appears to be highest among elderly patients [18, 20].

The granulocytic *Ehrlichia* agent, *B. microti,* and *Borrelia burgdorferi* share the same tick vector *(Ixodes scapularis),* and small rodents are the principal reservoir for all 3 agents [21–23]. Coinfection with these agents in both ticks and humans has been described [24–27]. Other unidentified enzootic organisms may also occur in a tick-rodent cycle. For example, a *Bartonella*-like agent has been identified in the blood of mice with concurrent *B. burgdorferi* or *B. microti* infection [28], although human infection with this agent has not been reported.

Because at least 3 tickborne infections can cause nonspecific febrile illnesses in Wisconsin, we undertook a prospective study to evaluate patients who have unexplained febrile illness without erythema migrans. The goals of the study were to (1) determine the proportion of unexplained febrile illnesses caused by *B. burgdorferi, B. microti,* or the HGE agent, (2) identify other novel bacterial pathogens by means of broad-range PCR, and (3) identify clinical or demographic characteristics associated with tickborne infection among patients with unexplained febrile illness.

PATIENTS AND METHODS

Ten primary care clinic sites were selected in northwestern Wisconsin for enrollment of patients with unexplained febrile illnesses. Participating clinic sites included the Marshfield Clinic regional centers in Ladysmith, Bruce, Cornell, and Rice Lake; Mayo-Midelfort Clinic sites in Barron, Cameron, Chetek, and Menominee (Red Cedar Clinic); and the Northwoods Community Health Center sites in Minong and Hayward. Sites were selected on the basis of location within an area with a high incidence of Lyme disease (>30 cases per 10^5 person-years) [29] and willingness to participate. Meetings were held with clinicians at these sites in spring 1997 to review the study procedures and eligibility criteria. Enrollment procedures and informational brochures were provided to each clinic, and phlebotomy kits were provided to the laboratory staff to facilitate proper specimen collection. Enrollment occurred from mid-May through August of both 1997 and 1998, and participating clinics were contacted at regular intervals (usually every 2 weeks) with reminders to enroll eligible patients.

Patients were eligible to be enrolled in the study if they had an acute, community-acquired febrile illness with a temperature >38.0°C, measured at home or in the clinic. Exclusion criteria included an illness with rash; localized source of infection evident on examination (e.g., otitis media, cellulitis, or pharyngitis); bacterial or parasitic etiology, documented by means of diagnostic testing (e.g., urinalysis, stool culture, or blood culture); antibiotic use in the past 7 days; institutional residency; and immunosuppressive condition. In addition, children <5 years old were excluded from enrollment.

When an eligible patient presented for medical care, the treating physician completed a case-enrollment form and a blood sample was obtained for diagnostic tests. Each participant (or parent of the participant) was interviewed by telephone to complete a standardized questionnaire regarding symptoms and tick exposure. A convalescent serum specimen was requested for serological testing at least 1 month after the onset of acute illness. Serological and other diagnostic test results were provided to the enrolling physician for each patient.

Whole blood, serum, and blood smears were forwarded to the Marshfield Laboratories for diagnostic testing. Acute serum samples were evaluated for IgM antibodies to *B. burgdorferi* by use of immunofluorescent assay (IFA) as described elsewhere [30]. At the Marshfield laboratories, the specificity and sensitivity of the IgM IFA are 100% and 42%, respectively, for patients with culture-confirmed primary erythema migrans [30]. A polyvalent enzyme immunoassay (General Biometrics) was also performed on acute and convalescent specimens [31]. All convalescent serum samples were also tested for IgG antibodies to *B. burgdorferi* by Western immunoblotting (MarDx Diagnostics), and blot results were interpreted according to criteria of the Centers for Disease Control and Prevention (CDC) [32].

A polyvalent IFA was performed on acute and convalescent serum samples by use of *B. microti* substrate (MRL Diagnostics) and fluorescein isothiocyanate–conjugated goat anti-human immunoglobulin (Kallestad Diagnostics), diluted 1:100. A titer $\geq$1:32 was defined as a positive result.

Wright-stained peripheral blood smears performed during the acute illness were examined for the presence of *Ehrlichia* inclusions (morulae) and *Babesia* piroplasms. Polyvalent IFA was performed on acute and convalescent serum samples by use of *E. equi* substrate (ProtaTek International) and fluorescein isothiocyanate–conjugated goat anti-human immunoglobulin (Kallestad Diagnostics), diluted 1:100. A titer $\geq$1:64 was defined as a positive result.

For PCR testing, DNA was extracted from blood by use of the Isoquick nucleic acid extraction kit (Microprobe). DNA was amplified in a standard PCR with use of primers Ehr 521 and Ehr 747. Specificity of PCR products was confirmed by means of Southern hybridization with a chemiluminescent internal probe generated by reamplification of a positive control specimen with primers Ehr 552 and Ehr 706 [33, 34].

For broad-range PCR, extracted DNA was amplified with prokaryotic broad-range PCR primers FD1/RD1 and 515/RD1 to recognize conserved bacterial 16S rRNA gene sequences [35]. PCR products were then sequenced with nested sequencing

primers by use of a cycle sequencing kit (Thermo Sequenase; Amersham Pharmacia) in an automated ALFexpress DNA sequencer (Amersham Pharmacia).

For this study, we defined "probable Lyme disease" as a febrile illness associated with an IgM-positive IFA of the acute sample or seroconversion with a positive IgG immunoblot of the convalescent sample. HGE was defined according to the CDC case definition [36]. Cases were confirmed on the basis of any of the following laboratory criteria for patients with febrile illness: 4-fold change in *E. equi* antibody titer, positive result of a PCR assay for the granulocytic *Ehrlichia* genogroup, or the combination of intracytoplasmic morulae and an IFA antibody titer $\geq$1:64. The finding of intracytoplasmic morulae only or a single IFA titer $\geq$1:64 was considered indicative of probable HGE.

Results were entered into the database and analyzed by use of SAS software, version 6.12 (SAS Institute). All laboratory results and the data from 20% of interview forms were entered in duplicate for quality assurance. Differences in categorical variables were analyzed by means of the χ^2 test or Fisher's exact test, as appropriate. Continuous variables were compared by use of the Wilcoxon 2-sample test.

RESULTS

A total of 91 patients with unexplained febrile illnesses were initially enrolled in the study from May through August of both 1997 and 1998. Twenty-nine patients were excluded because they had a rash illness (18 patients) or because acute and convalescent blood samples were not obtained (11 patients). Among the remaining 62 patients, the median age was 50 years (range, 8–85 years); all were non-Hispanic white patients. In addition to fever, the most common symptoms were fatigue, headache, myalgias, arthralgias, and chills. The median duration of illness prior to enrollment was 3 days. Eight patients (13%) were hospitalized and 22 (79%) were treated with an antibiotic.

Seventeen (27%) of 62 patients had laboratory evidence of tickborne infection, including 7 (11%) with probable Lyme disease only, 8 (13%) with HGE only, and 2 (3%) with apparent coinfection. None of the patients had seroconversion to positivity for *B. microti,* and piroplasms were not observed in any peripheral blood smears. Broad-range PCR of whole blood did not identify any other bacterial pathogens in participating patients.

The result of an acute titer of IgM to *B. burgdorferi* was positive in 8 of 9 patients with probable Lyme disease (including 2 patients with apparent coinfection); seroconversion to positivity for *B. burgdorferi* was detected in 3 patients with probable Lyme disease. The result of acute-phase EIA was positive for 4 patients with probable Lyme disease, including 2 patients whose IgG immunoblot result was positive.

The 10 HGE cases included 9 that met the criteria for confirmed HGE and 1 of probable HGE. PCR revealed rDNA of the HGE agent in 8 patients, and 6 had a 4-fold change in *E. equi* antibody titer. Seroconversion or seroreversion was detected in 5 of the 8 patients for whom the results of PCR were positive. One additional PCR-positive patient had a persistently high titer without seroreversion. The geometric mean convalescent titer was 1:256 (range, <1:32 to 1:2048). Characteristic granulocytic inclusions (morulae) were detected in the peripheral blood smear of 1 patient. For patients with HGE, the median platelet count was 121×10^3 platelets/μL (normal range, 175–450×10^3 platelets/μL), and the median leukocyte count was 4.5×10^3 cells/μL (normal range, 4.1–10.9×10^3 cells/μL).

At the time of enrollment, the clinicians for 9 patients (15%) indicated that the patients were "very likely" to have HGE, and 5 of these patients met the HGE case definition (positive predictive value, 56%). There were 3 cases that met the HGE case definition among 28 patients who were considered "somewhat likely" to have HGE. There were no cases of HGE among 17 patients who were considered "unlikely" or "somewhat unlikely" to have HGE (negative predictive value, 100%).

There were no clinical, demographic, or behavioral characteristics that distinguished patients with from patients without tickborne illness. The patients in the 2 groups were similar with regard to age, sex, symptoms, number of hours spent in woods or fields each week, and number of hours spent outdoors in their own yards each week (table 1). Tick bites were frequently reported by patients, but such reports were not significantly associated with tickborne illness. The positive predictive value of a recognized tick bite was 34%, and the negative predictive value was 79%.

DISCUSSION

The results of this study suggest that tickborne infections are an important cause of nonspecific febrile illness during the tick season in areas of Wisconsin where Lyme disease is endemic. We found serological evidence of acute Lyme disease in 9 (15%) of 62 eligible participants. Although these 9 patients did not have erythema migrans, it is likely that all had acute *B. burgdorferi* infection. Findings of previous studies at this institution suggest that the specificity of the IgM IFA test is nearly 100%, and the probability of a false-positive result is low [30]. The sensitivity of the IgM IFA test is <50%, and false-negative results may have occurred for some patients. As a result, the proportion of nonspecific febrile illnesses caused by Lyme disease may exceed 15% in northwestern Wisconsin during the tick season. This is comparable to the findings of a study from Connecticut, in which 5 (21%) of 24 patients with a nonspecific febrile illness (without erythema migrans) were found to have *B. burgdorferi* infection [3]. Two other prospective studies have suggested that 8%–17% of patients with Lyme disease will have a history of a flu-like illness without erythema migrans [4, 5]. Investigators

Table 1. Demographic, clinical, and behavioral characteristics of 62 patients with unexplained febrile illness during the tick season in northwestern Wisconsin.

Characteristic	Patients with tickborne illness (n = 17)	Patients without tickborne illness (n = 45)	P
Male	13 (76.5)	28 (62.2)	.38
Age, y			
5–19	2 (11.8)	4 (8.9)	.69
20–44	5 (29.4)	12 (26.7)	
45–69	7 (41.2)	25 (55.6)	
⩾70	3 (17.7)	4 (8.9)	
Month of onset			
May	3 (18.8)	6 (13.3)	.90
June	6 (37.5)	17 (37.8)	
July	4 (25.0)	15 (33.3)	
August	3 (18.8)	7 (15.6)	
Exposure history			
Occupational exposure to tick habitat	4 (23.5)	6 (13.3)	.44
Recognized tick bite	10 (58.8)	19 (43.2)	.39
Time spent outdoors, median			
In yard	8 h/w	11 h/w	.39
In tick habitat	7 h/w	5 h/w	.43
Acute symptoms			
Fatigue	15 (88.2)	38 (84.4)	1.0
Headache	14 (87.5)	41 (91.1)	.65
Myalgia	16 (94.1)	37 (82.2)	.42
Chills	14 (82.4)	34 (75.6)	.74
Arthralgias	16 (94.1)	32 (71.1)	.09
Abdominal pain	5 (31.3)	17 (37.8)	.77
Dyspnea	6 (35.3)	16 (35.6)	1.0
Cough	7 (41.2)	12 (26.7)	.36
Nasal congestion	8 (47.1)	11 (24.4)	.12
Sore throat	3 (18.8)	8 (17.8)	1.0
Hospitalized	4 (23.5)	4 (8.9)	.20

NOTE. Data are no. (%) of patients, unless otherwise indicated.

in these 2 studies used serological criteria for the diagnosis of Lyme disease. However, there is at least 1 case report of a patient with a nonspecific febrile illness who had *B. burgdorferi* isolated from blood but who did not have erythema migrans [37].

HGE was an important cause of unexplained fever in this study, accounting for 13% of cases during the tick season. Similar findings were found in a study of human monocytic ehrlichiosis as a cause of febrile illness at 3 Vanderbilt University hospitals [38]. In that study, 7 (18%) of 38 patients who presented with acute febrile illness and who had a history of tick bite had *Ehrlichia chaffeensis* isolated from samples of blood or CSF. For all 7 patients, the results of PCR tests with use of *E. chaffeensis*–specific primers were positive; 6 patients were seropositive.

For the current study, participating clinicians in Wisconsin received educational materials on HGE, and their clinical judgement regarding diagnosis of HGE was very good. No HGE occurred in cases classified by clinicians as "unlikely" to be HGE, and HGE was confirmed in more than half of the cases classified as "very likely" to be HGE. Overall, the results indicate that HGE should be considered along with Lyme disease in the differential diagnosis of nonspecific febrile illness in patients with an appropriate exposure history.

Babesia infections can cause severe illness in immunocompromised persons, but the majority of infections appear to be subclinical [8, 11, 12]. The absence of seroconversion among patients in this study suggests that *B. microti* is not a frequent cause of nonspecific febrile illness in Wisconsin. In addition, broad-range PCR testing did not identify any other bacterial infections caused by novel or known human pathogens.

The epidemiology and incidence of tickborne infections in the upper Midwest appear to differ from those in the northeastern United States [39, 40], and the results of this study may not be generalizable to other regions. In addition, the high proportion of patients with evidence of tickborne infection in this study may have been partially attributable to selection bias. The participating clinicians knew that the goal was to detect tickborne pathogens, and they may have been more likely to enroll patients with a known or suspected tick bite. However, patients who reported a tick bite did not have a significantly higher risk of tickborne infection than did patients without a known tick bite.

Most patients with early Lyme disease have erythema migrans, and this facilitates early diagnosis and treatment. Recognition of early stage tickborne infection in the absence of erythema migrans is more difficult, and the best strategy for evaluation and management of nonspecific febrile illness remains uncertain. We did not identify any distinguishing clinical or demographic characteristics of patients with tickborne infection. Although a tick bite was frequently reported by patients with tickborne illness, the positive predictive value was low. In addition, the suboptimal sensitivity of serological tests performed during the acute illness limits their utility for treatment decisions.

There is insufficient evidence to allow recommendation of routine empirical antibiotic treatment for patients with exposure to a tick habitat and acute febrile illness, but clinicians should consider the possibility of tickborne infection in this circumstance. Clinicians who treat patients for presumptive tickborne infection should use doxycycline rather than amoxicillin whenever possible, because the latter drug is ineffective against the HGE agent.

The recent introduction of a recombinant Lyme disease vaccine provides a new and possibly more effective strategy to reduce the occurrence of this disease [41]. However, the identification of HGE among patients with nonspecific febrile illness in Wisconsin highlights the continued importance of avoiding tick bites, regardless of Lyme disease vaccination status. Personal protective measures should include wearing long pants, using insect repellant containing DEET or permethrin, and performing daily skin checks following exposure to a tick habitat. Individuals who develop a febrile illness following exposure to a tick habitat should seek medical care promptly.

Acknowledgments

We are grateful to the following individuals for their contributions to the study: Angela Bender, Marilyn Daul, Juanita Herr, Shannon Meddaugh, and Katherine Nieman. We also thank the clinicians and staff at the following sites for their cooperation and participation: Marshfield Clinic regional centers in Ladysmith, Rice Lake, Cornell, and Bruce; Northwoods Community Health Center; Red Cedar Clinic; and the Mayo-Midelfort Clinic sites in Barron, Cameron, and Chetek.

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