

Clinical and Epidemiological Features of Early Lyme Disease and Human Granulocytic Ehrlichiosis in Wisconsin

Edward A. Belongia,¹ Kurt D. Reed,¹ Paul D. Mitchell,¹
Po-Huang Chyou,¹ Nancy Mueller-Rizner,¹
Michael F. Finkel,² and Martin E. Schriefer³

From the ¹Marshfield Medical Research Foundation, Marshfield Clinic, Marshfield, and ²Mayo-Midelfort Clinic, Eau Claire, Wisconsin; and ³Bacterial Zoonoses Branch, Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado

To compare clinical features and assess risk factors for human granulocytic ehrlichiosis (HGE) and early Lyme disease, we enrolled patients in a case-control study during the 1996 and 1997 tick seasons. Clinical and demographic characteristics were assessed for patients with laboratory-confirmed cases of HGE or Lyme disease, and risk factors were compared with those of matched control subjects. We identified 83 persons with Lyme disease, 27 with HGE, and 11 with apparent coinfection. Unsuspected *Ehrlichia* infection was identified in 8 (13%) of 60 patients with Lyme disease. Patients with HGE were older and more likely to have fever, chills, or dyspnea than were those with Lyme disease only. Most patients with apparent coinfection did not have hematologic abnormalities. In the risk factor analysis, tickborne illness was independently associated with rural residence and camping. The clinical spectrum of HGE overlaps that of Lyme disease, and physicians in areas of endemicity should consider both diseases in treating patients with a compatible rash or febrile illness.

Lyme disease is the most common vectorborne disease in the United States, and Wisconsin ranks eighth among states with the highest incidence of reported cases [1]. Human granulocytic ehrlichiosis (HGE) is another tickborne infection that was first described among residents of Wisconsin and Minnesota in 1994 [2]. HGE typically presents as an acute, nonspecific febrile illness, often accompanied by thrombocytopenia, leukopenia, and mild elevations of hepatic enzymes. The HGE agent and *Borrelia burgdorferi*, the cause of Lyme disease, share the same tick vector (*Ixodes scapularis*), and small rodents are the likely principal reservoir for both pathogens [3–5]. Culture-confirmed coinfection with *B. burgdorferi* and the HGE agent has been documented in humans [6].

Both Lyme disease and HGE can cause constitutional symptoms, but their clinical features have not been directly compared. In addition, little is known regarding the clinical significance of coinfection. To identify distinguishing clinical characteristics and assess risk factors, we prospectively identified cases of suspected early Lyme disease or HGE and offered enrollment in a case-control study.

Methods

Patients. Patients were eligible for enrollment if they were diagnosed with erythema migrans and/or suspected HGE from May 1996 through September 1997. The diagnosis of erythema migrans was based on clinical judgement, although clinicians were asked to report whether the rash was typical (i.e., annular erythema with central clearing) or atypical. Children aged <5 years and nonresidents of Wisconsin were excluded. Clinicians in the Marshfield Clinic regional health care system and the Mayo-Midelfort Clinic system enrolled most patients. During 1997, we also conducted active and laboratory-based surveillance to identify cases of suspected HGE in a 13-county area of northwestern Wisconsin. Case-finding was supplemented by weekly searches (during the tick season) of the computerized Marshfield Clinic medical record system, to identify all outpatients and inpatients with an ICD-9 (International Classification of Diseases, 9th edition) diagnosis of Lyme disease (088.81) or tickborne rickettsia or ehrlichiosis (082.8). For patients not already enrolled, the physician was contacted or the medical record was reviewed to determine eligibility.

Each enrolled patient completed a structured telephone interview regarding symptoms and potential risk factors for tickborne illness. The latter included residential environment, outdoor activities, and frequency of use of insect repellent, protective clothing, and other personal protective measures. For each case patient, we attempted to enroll 1 matched control subject to complete the same interview. Case patients and control subjects were matched by sex, age (± 10 years for adults aged ≥ 18 years; ± 5 years for children aged 5–17 years), and telephone exchange. We randomly selected control subjects from the pool of ~598,000 living Wisconsin residents who had ever received medical care from the Marshfield Clinic regional network; those with a self-reported history of Lyme disease during the same calendar year were excluded. Interviews of control subjects were always completed within 15 days of the matched case

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

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Reprints or correspondence: Dr. Edward A. Belongia, Epidemiology Research Center (ML2), Marshfield Medical Research Foundation, 1000 North Oak Ave., Marshfield, WI 54449 (belongie@mfldclin.edu).

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patients' interview date. The reference period for activities and exposures was 30 days before the onset of symptoms in the case-patient.

Diagnostic testing. All enrolled patients provided an acute blood sample for a complete blood cell count, measurement of alanine aminotransferase, and diagnostic tests for tickborne pathogens as described below. We requested a convalescent sample at least 1 month after enrollment for serological testing for Lyme disease and HGE. Skin biopsies were not routinely obtained for this study, although some clinicians elected to obtain a biopsy for culture of *Borrelia*.

Acute serum samples were evaluated for IgM antibodies to *B. burgdorferi* by immunofluorescent assay (IFA), as described elsewhere [7]. At Marshfield Laboratories, the sensitivity of the IFA for IgM is comparable to the Western blot for IgM in patients with culture-confirmed erythema migrans (unpublished data, P.M.). A polyvalent EIA (General Biometrics, San Diego, CA) was also used to test acute specimens [8]. All convalescent serum samples were tested for IgG antibodies to *B. burgdorferi* by Western immunoblot, and blot results were interpreted according to Centers for Disease Control and Prevention (CDC) criteria [9]. Marshfield Laboratories performed all serological tests, except the immunoblots for IgG on convalescent specimens, which were done by CDC.

Wright-stained peripheral blood smears obtained during the acute illness were examined for the presence of neutrophilic inclusions (morulae). We performed polyvalent IFA on acute and convalescent sera using *Ehrlichia equi* substrate (ProtaTek International, St. Paul, MN) and fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin (Kallestad Diagnostics, Chaska, MN) diluted 1 : 100. Titers $\geq 1 : 64$ were defined as positive according to CDC criteria [10].

For PCR testing, DNA was extracted from blood by means of the Isoquick nucleic acid extraction kit (Microprobe, Bothell, WA). DNA was amplified in a standard PCR that used primers Ehr 521 and Ehr 747 [4]. Specificity of PCR products was confirmed by Southern hybridization with a chemiluminescent internal probe generated by reamplification of a positive control specimen with primers Ehr 552 and Ehr 706. The specificity of this PCR assay in human samples appears to be high [11], and DNA sequencing at this laboratory has consistently confirmed the HGE agent in specimens from case patients that tested positive by PCR (unpublished data, K.R.).

Case definitions. We defined a case of early Lyme disease as a clinician-diagnosed erythema migrans in a patient with laboratory evidence of acute *B. burgdorferi* infection. The latter included a positive result on IFA testing for IgM in the acute sample, or seroconversion with a negative polyvalent EIA result in the acute sample and a positive result on immunoblot for IgG in the convalescent sample. In addition, a skin biopsy (if done) with culture isolation of *B. burgdorferi* was accepted as laboratory confirmation of Lyme disease.

HGE was defined according to the CDC case definition [10]. Confirmed cases were based on any of the following laboratory criteria in a patient with a clinically compatible illness: 4-fold change in *E. equi* antibody titer, positive PCR assay for the granulocytic *Ehrlichia* genogroup, or the combination of intracytoplasmic morulae and an IFA titer $\geq 1 : 64$. Patients with probable

cases of HGE were those with intracytoplasmic morulae only or a single IFA titer $\geq 1 : 64$ [10].

Statistical analyses. The Wilcoxon rank sum test for 2 independent samples was used for the comparison of continuous variables (age, total leukocyte count, platelet count, hemoglobin level, and alanine aminotransferase level) among case patients with Lyme disease, HGE, and apparent coinfection. The Fisher's exact test or χ^2 test was used, as appropriate, for comparison of categorical variables in the 3 groups of case patients.

For the study of matched case patients and control subjects, we assessed potential risk factors by use of univariate conditional logistic regression analysis, with calculation of matched ORs and 95% CIs [12]. Continuous variables were compared by the paired Student's *t* test or the Wilcoxon rank sum test. Potential risk factors with a univariate *P* value of $\leq .10$ were entered into a multivariate conditional logistic regression model by use of a forward stepwise selection process. Age was included as a potential covariate in the model. The population attributable risk was calculated on the basis of the adjusted OR and the proportion of case patients reporting the risk factor [13]. All reported *P* values are 2-sided, and *P* $\leq .05$ was considered statistically significant. Statistical analyses were done with SAS version 6.12 (SAS Institute, Cary, NC).

Results

A total of 297 patients were enrolled in the case-control study from May 1996 through September 1997. We excluded 14 (4.7%) from the analyses because the patient did not meet eligibility criteria (*n* = 11), withdrew from the study (*n* = 2), or because a matching control subject could not be identified (*n* = 1). Of the remaining 283 patients, 83 (29%) had Lyme disease only, 27 (10%) had confirmed HGE only, and 11 (4%) had both Lyme disease and confirmed (*n* = 8) or probable (*n* = 3) HGE. One (0.35%) was classified as having probable HGE only. The remaining 161 patients did not meet any of the case definitions and were excluded from further analyses, although 109 (65%) had a rash illness that may have represented seronegative Lyme disease.

Further analyses were limited to the 121 patients with confirmed HGE or Lyme disease, including the 11 patients with apparent coinfection; 118 (98%) were non-Hispanic white, and 65 (54%) were employed. The onsets of tickborne illness occurred from April through October, with 87 (72%) occurring in June or July. The median interval from symptom onset to phlebotomy was 8 days. Among 83 patients with Lyme disease only, erythema migrans was described as typical (annular erythema with central clearing) in 58 (70%) and atypical in 25 (30%). Among the 11 patients with apparent coinfection, erythema migrans was described as typical in 8 (73%) and atypical in 3 (27%). Patients with Lyme disease only were significantly younger than patients with HGE only (*P* = .004) or apparent coinfection (*P* = .012).

Patients with HGE only were significantly more likely to experience fevers, chills, and dyspnea than were those with Lyme disease only, but there was substantial overlap in symp-

toms (table 1). Unsuspected HGE was identified in 8 (13%) of 60 patients with erythema migrans who met the Lyme disease case definition. Patients with apparent coinfection (laboratory evidence of HGE with concurrent erythema migrans and laboratory evidence of *B. burgdorferi* infection) were significantly less likely to report fever, chills, and fatigue than were those with HGE only.

Both total leukocyte counts and platelet counts were similar in patients with Lyme disease only and in those with apparent coinfection, but both counts were significantly lower in patients with HGE only. The platelet count was low ($< 175 \times 10^3$ cells/ μL) in 8 (11%) of 75 patients with Lyme disease only, 19 (79%) of 24 patients with HGE only, and 1 (14%) of 7 with apparent coinfection (data not available for the remaining patients). Leukopenia ($< 4.1 \times 10^3$ cells/ μL) was present in 2 (3%) of 74 patients with Lyme disease, 6 (26%) of 23 with HGE only, and

none of 7 with apparent coinfection. However, 1 patient with apparent coinfection was hospitalized several days before enrollment, and her admission laboratory results demonstrated thrombocytopenia and leukopenia.

Since both HGE and Lyme disease are acquired from the bite of infected *I. scapularis* ticks, we combined these diagnoses for the analysis of potential risk factors. The mean intervals from the date of case diagnosis to completion of the telephone interview were 3.2 days for case patients and 8.3 days for matched control subjects. Case patients and matched control subjects were similar with regard to race, education, and employment status.

The following residential factors were associated with tick-borne infection by univariate analysis: residence outside the borders of a town or city, rural neighborhood (vs. urban or subdivision), property size >2 acres, residence on or bordering

Table 1. Demographic, clinical, and laboratory characteristics of case patients in a study of Lyme disease and human granulocytic ehrlichiosis (HGE).

Characteristic	Lyme disease only (n=83)	HGE only (n=27)	Apparent coinfection (n=11)	P, Lyme vs. HGE ^a	P, Lyme vs. coinfection ^b	P, HGE vs. coinfection ^c
Age, y	42	53	54	.004	.012	.797
Male	53 (64)	18 (67)	5 (45)	1.0	.324	.285
Initial clinical diagnosis						
Suspected Lyme disease only	52 (63)	1 ^d (4)	7 (64)	.001	1.0	.001
Suspected HGE only	0	23 ^d (88)	0			
Both	31 (37)	2 ^d (8)	4 (36)			
Acute symptoms						
Fever	58 (70)	25 (93)	6 (54)	.020	.320	.014
Chills	56 (67)	24 (92)	6 (54)	.011	.501	.016
Headache	70 (84)	20 (74)	5 (45)	.256	.008	.135
Fatigue	70 (84)	25 (93)	7 (64)	.351	.108	.047
Myalgias	63 (76)	19 (70)	7 (70)	.614	.704	1.0
Arthralgias	57 (69)	15 (56)	6 ^d (60)	.248	.722	1.0
Dyspnea	14 (17)	14 (52)	2 (18)	<.001	1.0	.078
Hospitalized	5 (6)	11 (41)	1 (9)	<.001	.536	.121
Total leukocyte count $\times 10^3/\mu\text{L}^e$	7.2 (3–20.2)	5.7 (1.4–11.7)	8.2 (6.3–9.9)	<.001	.250	.001
Platelet count $\times 10^3/\mu\text{L}^f$	238 (94–599)	112 (8–242)	236 (121–322)	<.001	.771	.003
Hemoglobin level, g/dL ^g	13.7 (10.7–17.4)	14.3 (9.7–18.6)	13.7 (12.3–15.6)	.327	.712	.478
ALT level, U/L ^h	28 (7–327)	60 (9–260)	40 (13–90)	<.001	.344	.166
Lyme disease test results						
Acute sample, positive IgM antibody titer	75 (90)	0	7 (64)	—	—	—
EIA seroconversion	10/75 (13)	0	0	—	—	—
Convalescent sample, Western Blot for IgG ⁱ	33/75 (44)	5/25 (20)	6 (55)	—	—	—
HGE test results						
Smear positive (morulae)	—	15 (56)	1	—	—	—
PCR positive	—	17/24 (71)	2 ^d (20)	—	—	—
Antibody to <i>Ehrlichia equi</i>						
Seroconversion/seroreversion	—	19 ^d (73)	7 (64)	—	—	—
≥ 1 finding of elevated titer	—	22 (81)	9 (82)	—	—	—

NOTE. Data are no. (%), or no./total tested (%), or median (range). CDC, Centers for Disease Control and Prevention.

^a Lyme disease only vs. HGE only.

^b Lyme disease only vs. apparent coinfection.

^c HGE only vs. apparent coinfection.

^d Data not reported for 1 patient.

^e Leukocyte count was not available for 9 case patients with Lyme disease only, 4 case patients with HGE only, and 4 patients with apparent coinfection. Normal values, $4.1\text{--}10.9 \times 10^3/\mu\text{L}$.

^f Platelet count was not available for 8 case patients with Lyme disease, 3 case patients with HGE, and 4 patients with apparent coinfection. Platelet count was reported as range for 5 patients; thus, midpoint was used to estimate actual value. Normal values, $175\text{--}450 \times 10^3/\mu\text{L}$.

^g Normal values: men, 12.9–17.3 g/dL; women, 11.7–15.5 g/dL.

^h Alanine aminotransferase. Normal values: men, 6–47 u/L; women, 5–35 u/L.

ⁱ Meeting the criteria of the Centers for Disease Control and Prevention

farm acreage, and a dog in the household. There was a significant correlation between property size >2 acres and rural neighborhood (Spearman correlation, $r = .53$; $P < .001$). Residential factors that were not associated with tickborne illness included proximity to woods, frequency of deer sighting, undeveloped yard, presence of gardens or woodpiles in the yard, or a cat in the household.

Behavioral factors associated with tickborne illness in the univariate analysis ($P < .10$) included camping, clearing brush on and off the property, hiking unpaved trails, jogging, recognized tick bite, and finding ticks on clothing, hair, or skin. Case patients did not have an increased odds of occupational exposure to woods, fields, or other undeveloped areas. There was no significant difference in the amount of time spent outdoors in woods or fields each week.

To identify environmental and behavioral factors associated with tickborne illness, we created an initial multivariate model that excluded the tick bite and tick-exposure variables. In this model, only rural neighborhood and camping were significantly associated with tickborne illness (table 2). The population attributable risk was highest for rural neighborhood, accounting for more than half of all cases. When the multivariate model was repeated with the inclusion of recognized tick bite and tick exposure (on hair, skin, clothing) as potential risk factors, the following variables were significantly associated with tickborne illness: recognized tick bite, jogging, clearing brush off property, camping, and property size >2 acres (table 3). We were unable to perform a separate analysis of risk factors for children, because only 24 cases were identified among children aged <18 years.

There were 47 (39%) case patients who recalled a tick bite. Nineteen (49%) of 39 were <100 yards from home and 10 (26%) were at least 1 mile from home when the tick bite was acquired (not reported for 8 patients). Among case patients with a recognized tick bite, the median number of tick bites during the 30 days before symptom onset was 1 (range, 1–20 bites). The tick bite characteristics could not be compared with those of control subjects, because there were only 9 matched pairs in which both the case patient and the control subject reported a tick bite.

The use of insect repellent and other outdoor personal protection behaviors was similar among case patients and control subjects (data not shown). Both case patients and control subjects had high scores for wearing long pants and checking for ticks after outdoor activity, indicating that these protective measures were used frequently. The frequency of use was lowest for insect repellent and tucking in pant legs. For the 65 case-control pairs who lived in a rural area, there was a weak association between tickborne illness and less frequent use of long pants ($P = .047$) and less frequent use of insect repellent on skin ($P = .113$) or clothing ($P = .057$).

To evaluate the possibility that the control population was biased toward persons with chronic illness, we compared the

Table 2. Results of multivariate logistic regression model and estimated population attributable risk for behavioral and environmental factors associated with tickborne illness.

Risk factor	Adjusted matched OR	95% CI	P	Case patients reporting, %	Population attributable risk, %
Rural neighborhood	3.3	1.6–6.8	<.001	17.5	58
Camping	3.0	1.3–6.7	.006	23.1	15

NOTE. Tick bite and tick exposure variables were not included in this model.

prevalence of heart disease, cancer, stroke, and diabetes in the control group with the same prevalences in residents of the Marshfield Epidemiologic Study Area, a defined population of ~83,000 persons living in 22 Wisconsin zip codes [14]. The age- and sex-adjusted prevalence of these chronic diseases was similar among the control subjects (18.6%) and among residents of the Marshfield Epidemiologic Study Area (16.9%; $P = .370$).

Discussion

The results of this study demonstrate that the clinical characteristics of HGE and early Lyme disease overlap substantially. Since the conditions may be acquired simultaneously, *Ehrlichia* infection should be suspected in patients with erythema migrans in the upper Midwest. In particular, older patients with early Lyme disease should be screened for hematologic abnormalities, since the clinical severity of HGE increases with age [15]. Those with thrombocytopenia or leukopenia require additional diagnostic tests to confirm HGE while treatment is initiated. Although doxycycline is the recommended therapy for both Lyme disease and HGE, patients with hematologic abnormalities require close monitoring for complications and response to therapy.

Patients with laboratory evidence of coinfection represented 9% of all patients with Lyme disease or confirmed HGE, and all had clinician-diagnosed erythema migrans with positive results of serological testing for Lyme disease. These patients were significantly older than those with Lyme disease only, but most lacked the typical hematologic abnormalities associated with HGE. There are several possible explanations for these findings. The clinical spectrum of HGE may include milder illness that was detected only because diagnostic tests for *Ehrlichia* were performed for a large group of patients with erythema migrans. This is suggested by 2 patients who had *Ehrlichia* DNA detected by PCR, although their total leukocyte counts and platelet counts were normal. False-positive *E. equi* serology is another possible explanation for some cases of apparent coinfection, particularly the 2 cases that were diagnosed on the basis of an elevated HGE antibody titer without seroconversion. There have been no published reports of false-positive serological tests for HGE among patients with Lyme disease, but the potential for false-positive results is apparent from the recent observation that nearly 15% of asymptomatic adults in northwest Wisconsin have antibodies indicative of HGE [16].

Table 3. Results of conditional logistic regression model and estimated population attributable risk with inclusion of tick bite and tick exposure variables.

Risk factor	Adjusted matched		P	Case patients reporting, %	Population attributable risk, %
	OR	95% CI			
Jogging	48.6	2.4–998.9	.002	5.8	6
Clearing brush	6.1	1.1–33.2	.016	13.2	11
Recognized tick bite	3.5	1.6–7.8	<.001	42.0	30
Camping	2.7	1.0–6.9	.034	23.1	15
Property size >2 acres	2.1	1.1–4.4	.013	35.3	18

It is difficult to distinguish sequential tickborne infections from synchronous infections unless both infections are confirmed by culture or PCR. In this study, some patients with apparent coinfection may have had tickborne diseases that were acquired sequentially rather than simultaneously. In both circumstances, a 4-fold change in antibody titer may be detected. If the first tickborne infection causes immunologic priming, it might reduce the severity of a subsequent infection with a different pathogen. However, there is currently no evidence for this phenomenon with tickborne pathogens. Further studies are needed that use PCR or culture to assess the clinical significance and frequency of sequential infection versus simultaneous coinfection.

In the analysis of risk factors, we found that camping, clearing brush off property, jogging, property size >2 acres, and a recognized tick bite during the month before symptom onset were all significantly associated with incident tickborne illness. Occupational exposure to tick habitat was not identified as a risk factor. When we excluded tick bite and tick exposure (i.e., finding ticks on skin or clothing) from the logistic regression model, only rural residence and camping during the month before onset were significantly associated with tickborne illness. Rural residence has been identified as a risk factor for tickborne illness in the eastern United States [17, 18], but there appear to be regional variations in other risk factors. Suburban development and residential property characteristics such as rock walls, woods, and deer contribute to Lyme disease risk [17, 19] in the eastern United States, but these residential characteristics appear to be less important in northwestern Wisconsin. Regional data on the frequency and types of specific outdoor recreational activities are not available, but <5% of Lyme disease patients in New Jersey went camping, compared with 23% of case patients in Wisconsin [17]. Therefore, the attributable fraction for different outdoor activities may be different in the upper Midwest than in the eastern United States.

Insect repellent containing 20% or 30% deet (*N,N*-diethylmeta-toluamide) is an effective tick repellent when sprayed on clothing for 1 minute [20]. However, the effectiveness may be reduced if the product is sprayed on unevenly or for shorter time periods. We were unable to document the overall effectiveness of insect repellent use or other personal protective measures, although there may have been some benefit for rural residents. Results were inconsistent in 2 other studies [17, 21].

Overall, the available data suggest that protective measures are not widely used [22] and may not be highly effective, but they are easy to use and relatively safe, so should continue to be recommended.

The limitations of this study include the lack of standardized criteria for the clinical diagnosis of erythema migrans and the potential for recall bias among case patients. In addition, the large number of variables examined could have led to some significant findings by chance. For example, the association between tickborne illness and jogging was based on only 6 discordant pairs, resulting in a wide confidence interval.

Two different Lyme disease vaccines, both derived from recombinant outer surface protein A (OspA) antigen, have been evaluated in clinical trials, and 1 has been approved for use in adults by the US Food and Drug Administration [23, 24]. At this time, there are no national or state guidelines regarding the populations to be vaccinated, but it is likely that future guidelines will include risk assessment for persons living in areas of endemicity. The risk factors identified in this study and others may be useful for clinicians who counsel patients regarding the need for a Lyme disease vaccine.

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