CONCISE COMMUNICATIONS

The Incidence of Ehrlichial and Rickettsial Infection in Patients with Unexplained Fever and Recent History of Tick Bite in Central North Carolina

Christopher F. Carpenter,[1,a] Tejal K. Gandhi,[1,a] Li Kuo Kong,[1,a] G. Ralph Corey,[2] Sheng-Min Chen,[b] David H. Walker,[4] J. Stephen Dumler,[5] Edward Breitschwerdt,[3] Barbara Hegarty,[3] and Daniel J. Sexton[2]

[1]Department of Medicine and [2]Division of Infectious Diseases, Duke University Medical Center, Durham, and [3]Departments of Companion Animal and Special Species Medicine, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina; [4]Department of Pathology, University of Texas Medical Branch at Galveston, Galveston; [5]Department of Pathology, Division of Medical Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland

We examined the clinical and laboratory findings of a consecutive series of patients from central North Carolina presenting with fever and a history of tick bite within the preceding 14 days. Evidence of a tick-transmitted pathogen was detected in 16 of 35 patients enrolled over a 2-year period. Nine patients were infected with *Ehrlichia chaffeensis*, and 6 were infected with a spotted fever group rickettsia; 1 patient had evidence of coinfection with *E. chaffeensis* and a spotted fever group rickettsia. Four patients had detectable antibodies against the human granulocytic ehrlichiosis agent; however, only 2 had a 4-fold antibody titer rise without detectable antibodies against *E. chaffeensis*. The other 2 were thought to have cross-reacting antibodies to *E. chaffeensis*. We conclude that ehrlichial infections may be as common as spotted fever group rickettsial infections in febrile patients from central North Carolina with a recent history of tick bite.

Numerous viruses, bacteria, rickettsiae, spirochetes, and protozoa can be transmitted to humans by ticks. Tick-borne diseases endemic to the United States include Rocky Mountain spotted fever (RMSF), granulocytic and monocytic ehrlichiosis, babesiosis, tularemia, Colorado tick fever, and Lyme disease. In central North Carolina, RMSF has long been assumed to be the primary endemic tick-borne disease. For years it has been our standard clinical practice to presumptively treat all patients complaining of fever in association with a history of recent tick bite or tick exposure with tetracycline or doxycycline, yet only a minority of these patients were shown to have had infection with a spotted fever group rickettsia by serologic testing.

The recent recognition of infection with *Ehrlichia chaffeensis* (the cause of human monocytic ehrlichiosis [HME]) and with the human granulocytic ehrlichiosis (HGE) agent in patients from the eastern United States raised the possibility that many patients previously treated "successfully" for RMSF actually had ehrlichiosis. However, there are scant prospectively collected data on the relative frequency of these pathogenic agents in areas such as North Carolina. We undertook the following study of patients presenting with fever and a recent history of tick bite during the spring and summer months of 1995 and 1996 with the primary goal of determining the proportion of patients with rickettsial and/or ehrlichial infection.

Methods

We recruited adult patients (aged ⩾18 years) who presented to primary care and acute care facilities in Durham County during the months March–September over a 2-year period. To be eligible for the study, patients had to have a history of tick bite during the preceding 2 weeks and fever not explained by other clinical findings (e.g., urinary tract infection). Venous blood was drawn at the time of initial presentation, and a convalescent venous blood sample was obtained between 2 and 8 weeks after the onset of symptoms.

Received 4 January 1999; revised 10 May 1999; electronically published 9 August 1999.

Presented in part: 13th Sesquiannual Meeting of the American Society for Rickettsiology, Champion, PA, September 1997 (abstract 73).

Informed consent was obtained from all study participants; the Institutional Review Board of the Duke University School of Medicine approved the study.

[a] Present affiliations: Department of Medicine, Division of Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, Maryland (C. F. C.); Division of General Medicine, Brigham and Women's Hospital, Boston, Massachusetts (T. K. G.); Division of Infectious Diseases and Geographic Medicine, Stanford University Medical Center, Stanford, California (L. K. K.).

[b] Sheng-Min Chen, the first person to identify the agent of HGE and a promising young scientist, was killed in an automobile accident in January 1997. She is missed by her colleagues in rickettsiology.

Reprints and correspondence: Dr. Daniel J. Sexton, Box 3605, Duke University Medical Center, Durham, NC 27710 (sexto002@mc.duke.edu).

The Journal of Infectious Diseases 1999; 180:900–3

0022-1899/1999/18003-0050$02.00

Table 1. Results of diagnostic tests of 35 patients from central North Carolina with fever and recent tick bite.

Laboratory test	Results				
	R. rickettsii infection ($n = 5$)	*E. chaffeensis* infection ($n = 8$)	*E. chaffensis* and *R. rickettsii* coinfection[a] ($n = 1$)	HGE agent infection ($n = 2$)	Total ($n = 35$)[b]
⩾4-fold rise in *R. rickettsii* antibody titer	5	0	0	0	5
⩾4-fold rise in *E. chaffeensis* antibody titer or elevated acute antibody titer	0	8	1	0	9
⩾4-fold rise in HGE agent antibody titer or elevated acute antibody titer	0	2[c]	0	2	4
PCR positive for *E. chaffeensis*[d]	0/2	2/5[e]	1/1	0/1	3/21[e]

NOTE. HGE, human granulocytic ehrlichiosis; PCR, polymerase chain reaction; *R. rickettsii*, *Rickettsia rickettsii*; *E. chaffeensis*, *Ehrlichia chaffeensis*.

[a] The patient with coinfection had negative serology but positive culture and tissue immunofluorescence assay for *R. rickettsii.*

[b] Total includes 19 patients without evidence of ehrlichial or rickettsial infection.

[c] Both patients had *E. chaffeensis* infection confirmed by other methods. Elevated titers to the HGE agent were felt to be cross-reacting antibodies to *E. chaffeensis.*

[d] Not all patients were tested. Fractions are number positive/number tested in each category.

[e] One additional patient was found to have *E. chaffeensis* by PCR with eubacterial 16S rDNA primers and subsequent sequence analysis.

We obtained clinical data from the patient's physician, and we reviewed laboratory data if they were obtained at the time of the initial outpatient evaluation or during subsequent hospitalization.

Serology. Acute and convalescent serum samples were tested for antibodies to *E. chaffeensis,* the HGE agent, and *Rickettsia rickettsii* antigens by use of the indirect immunofluorescence antibody (IFA) test, as described elsewhere, except that *E. chaffeensis* (Arkansas strain) was used in place of *E. canis* [1–3]. Western blots were performed on selected sera with high titers against both ehrlichiae. For Western blots, density gradient–purified *E. chaffeensis* (Arkansas strain) and the HGE agent (Webster strain) were used as described elsewhere [4, 5].

Polymerase chain reaction (PCR) amplification. DNA amplification was performed by use of primers specific for either *E. chaffeensis* or the HGE agent, and amplification by use of eubacterial primers was also performed with subsequent sequence analysis of select positive samples for specific pathogen identification.

For *E. chaffeensis* PCR, DNA was extracted from acute-phase whole blood with a commercially available kit (Iso Quick; Orca Research, Bothell, WA) according to manufacturer's instructions. The dried pellet was then resuspended in DNase-free water in preparation for PCR analysis. Primers that recognized sequences of the *nadA* gene and the 120-kDa protein gene of *E. chaffeensis* were used to analyze the extracted DNA samples. The amplification conditions for the 120 kDA protein gene were essentially the same as the ones used for the *nadA* gene, except that nested PCR was performed [6]. The outside primers were pXCF3 and pXAR4, and the nested primers were pXCF3b and pXAR5. The nested primers produced a product similar to the 1.1-kb product amplified from the Arkansas strain and one repeat unit larger than the Sapulpa strain. PCR was performed at 30 cycles for 30 s at 94°C, 1 min at 55°C, and 2 min at 72°C. The PCR products were then separated by electrophoresis in a 1% agarose gel stained with ethidium bromide and visualized under UV light.

For HGE agent PCR testing, DNA in acute-phase blood was prepared by use of the Puregene kit (Gentra Systems, Minneapolis), and PCR was performed as described elsewhere by use of primers ge9f and ge10r [7].

For amplification of eubacterial 16S ribosomal RNA genes in acute-phase blood, DNA prepared by the Puregene kit was targeted as described elsewhere by use of primers EC12 and EC9 [8]. Sequence analysis was performed by use of a dideoxynucleotide cycle sequencing method and an Applied Biosystems (Foster City, CA) model 377 automated fluorescent sequencer.

Statistical analysis. Clinical laboratory values and categorical results were compared between diagnostic groups by two-sided t test with unequal variances and two-sided Fisher's exact test, respectively [9]. Results were considered statistically significant at $P < .05$.

Results

During the 2-year period of the study, 91 patients with suspected tick-borne disease were evaluated. Forty-eight had fever and a history of recent tick bite, and 35 patients agreed to participate in the study. Clinical data were obtained on all patients at the time of initial medical presentation, and laboratory data were obtained on 30 patients. The average age of enrolled patients was 46 years (range, 20–75). Thirty of 35 individuals were white, and 15 were male. The average interval from identification of tick bite to enrollment in the study was 3.7 days (range, 0–11 days; median, 5 days).

Serologic and PCR findings. Fourfold or greater rises in antibody titer against *R. rickettsii* antigens were detected by IFA testing in 5 (14%) of 35 patients (table 1). An additional patient, reported elsewhere [6], had negative antibody titers to *R. rickettsii* antigens but had a positive rickettsial blood culture and a skin biopsy that showed organisms in arterioles that stained with immunofluorescent conjugates against *R. rickettsii,* as well as evidence of *E. chaffeensis* infection by serology and PCR.

A 4-fold or greater rise in *E. chaffeensis* antibody titer was detected in 7 (20%) of 35 patients. Markedly elevated acute blood titers to *E. chaffeensis* were detected in 2 additional patients who did not have evidence of HGE or RMSF. Two pa-

tients had a 4-fold rise in antibody titers to the HGE agent (both <80–160), without evidence of RMSF or HME.

Elevated antibody titers against HGE agent antigen were also detected in 2 patients with serologic evidence of HME. However, further studies indicated that both patients actually had HME, with elevated HGE agent titers resulting from cross-reaction with *E. chaffeensis* antigen. One of these patients had a >10-fold rise in titer (<80–2560) to *E. chaffeensis* antigens and a corresponding rise in HGE agent antibody titer (80–640). He also had inclusions typical of *E. chaffeensis* in monocytes visualized on a peripheral smear, and eubacterial 16S rRNA gene sequences were amplified from his acute-phase blood sample by PCR. Sequence analysis of the PCR product revealed 100% identity to *E. chaffeensis* 91HE17 strain (GenBank accession no. ECU23503) in 1435 examined nucleotides. The other patient with elevated titers to the HGE agent (2560–5120) had at least a 2-fold rise in antibody titers to *E. chaffeensis* (5120–10,240). Western blot studies of this patient's acute and convalescent serum both showed a band in the 28–30 kDa region with *E. chaffeensis* antigens, but no band was identified in the 44 kDa region when tested with HGE agent antigens, findings most consistent with *E. chaffeensis* infection [3].

PCR amplification of *E. chaffeensis* DNA from blood samples containing EDTA was attempted in 21 patients; 3 of 21 patient samples contained *E. chaffeensis* DNA; all 3 of these PCR-positive patients had 4-fold rises in antibodies to *E. chaffeensis* by IFA testing. Three *E. chaffeensis*–seroreactive patients who were tested by PCR had negative results. Of the 4 patients tested by PCR for DNA from the HGE agent (including 1 of the patients with positive HGE agent serology), none were positive. Four patients were tested for eubacterial 16S rDNA by PCR, with 1 patient, noted above, having positive results with subsequent identification of *E. chaffeensis* by sequence analysis. The 3 patients with negative eubacterial PCR results had no other evidence of tick-borne infection.

Clinical findings. Rashes were observed at the time of presentation or subsequently in 12 (34%) of 35 patients, including 4 of 6 patients with *R. rickettsii* infection or coinfection (table 2). Three of 9 patients diagnosed with *E. chaffeensis* infection or coinfection had a rash. The 2 patients with a 4-fold antibody titer rise to HGE did not have a rash. All 35 patients were febrile, and all patients complained of myalgias and headache. Fifteen of 35 patients enrolled in the study were admitted to the hospital. Patients with infection caused by a spotted fever group rickettsia (5/5) or *E. chaffeensis* (5/8) were significantly more likely to be hospitalized than those who did not have a diagnosed cause of their fever (2/19; $P < .001$ and $P = .011$, respectively). The patient with coinfection also required hospitalization. The mean interval between onset of illness and the institution of therapy (doxycycline) was 4.3 days for patients with RMSF, 4.3 days for patients with ehrlichial infection, and 3.4 days in patients for whom a diagnosis was not established.

Patients diagnosed with infection caused by a spotted fever group rickettsia or *E. chaffeensis* had significantly lower average leukocyte cell counts (5.1 and 5.4×10^9/L, respectively), compared with those without a diagnosed cause of their fever (cell counts of 10.1×10^9/L, $P = .038$ and $P = .035$, respectively; note that the coinfected patient was not included in the statistical analysis for either group). The average leukocyte counts in those with *R. rickettsii* infection did not differ significantly from those with *E. chaffeensis* infection. Average platelet counts were significantly lower in patients with RMSF (70,000 cells/μL) than in those without a diagnosed cause of their fever (259,000 cells/μL, $P < .001$). The average platelet counts in those with *E. chaffeensis* (181,000 cells/μL) infection were not significantly different from either group. The average leukocyte count and platelet count for the 2 patients with HGE agent and the counts for the patient coinfected with a spotted fever group rickettsia and *E. chaffeensis* are listed in table 2. We did not find significant differences between diagnosis groups for serum sodium, creatinine, aspartate aminotransferase, or alanine aminotransferase (data not shown), although the small number of patients who had these tests performed, particularly in the nondiagnosed group, markedly limited statistical analysis.

Table 2. Results of select tests and clinical features for patients presenting with fever and recent tick bite in central North Carolina.

Pathogen (*n*)	Mean WBC count $\times 10^9$/L (SD)[a]	Mean platelet cell count/μL (SD)[b]	No. with rash (%)	No. hospitalized (%)
R. rickettsii (5)	5.1 (3.7)[c]	70,000 (53,000)[c]	3 (60)	5 (100)[d]
E. chaffeensis (8)	5.4 (4.7)[c]	181,000 (165,000)	2 (25)	5 (63)[d]
Coinfection with *R. rickettsii* and *E. chaffeensis* (1)	2.7	6900	1 (100)	1 (100)
Agent of human granulocytic ehrlichiosis (2)	5.8 (4.4)	121,000 (88,000)	0 (0)	1 (50)
Patients without evidence of ehrlichial or rickettsial infection (19)	10.1 (4.3)[e]	259,000 (72,000)[e]	6 (32)	3 (16)

NOTE. The patient coinfected with *Ehrlichia chaffeensis* (*E. chaffeensis*) and *Rickettsia rickettsii* (*R. rickettsii*) was not included in the statistical analysis of either group; *n*, number of patients in each pathogen category; WBC, white blood cell.

[a] Normal cell range: $3.2–9.8 \times 10^9$/L.

[b] Normal range: >150,000 cells/μL.

[c] Statistically significant difference from patients without evidence of ehrlichial or rickettsial infection ($P < .05$, two-sided *t* test).

[d] Statistically significant difference from patients without evidence of ehrlichial or rickettsial infection ($P < .05$, two-sided Fisher's exact test).

[e] $n = 14$. Five patients did not have clinical laboratory studies available.

Discussion

Most studies of humans with tick-borne diseases have been analyses of the clinical, serologic, or pathologic features of cases collected retrospectively from the records of large medical centers or public health laboratories. Only a few studies have prospectively collected data from a geographic area [10, 11]. Our study is unique in that it prospectively evaluated febrile patients with a recent tick bite and utilized modern laboratory techniques to confirm the diagnosis.

The results of our study were unexpected in several respects. The incidence of ehrlichial infection exceeded that of RMSF, as we found 8 patients with HME, 5 with RMSF, 1 with coinfection with *E. chaffeensis* and *R. rickettsii,* and 2 with HGE. Also, patients infected with *R. rickettsii* or *E. chaffeensis* had lower leukocyte counts and lower platelet counts than patients without a diagnosed cause of their fever. The comparison of laboratory values between patients with infections caused by *R. rickettsii* and *E. chaffeensis* was less revealing because of the low number of patients diagnosed, but we suspect that there may be lower platelet counts and higher transaminase elevations (data not shown) in patients with RMSF.

We and others have reported that the early institution of antimicrobial therapy may blunt or prevent the development of antirickettsial antibodies in convalescent serum samples from patients with RMSF [6, 12]. Because most of our patients were treated soon after the onset of symptoms (mean, 3.7 days) and because rickettsial cultures and PCR methods were not used to detect the presence of *R. rickettsii* or ehrlichiae in all patients, it is possible that some patients who were treated early and had negative diagnostic studies actually had RMSF or ehrlichiosis.

Two patients had a 4-fold antibody titer rise to the HGE agent antigens without evidence of RMSF or HME. This finding was a surprise to us, as the presumed vector for the HGE agent, *Ixodes scapularis,* is not thought to be abundant in central North Carolina (N. MacCormack, personal communication). Results of seroprevalance studies in Wisconsin suggest that 15% of the population is seropositive, with a mean geometric titer of 250 [13]. However, as a 4-fold antibody titer rise against the HGE agent occurred in our patients, we believe that these titers represent acute infection rather than prior exposure or a false-positive result. In two other cases, antibodies to HGE agent antigens were thought to be due to *E. chaffeensis* cross reactivity. Other authors have reported the occurrence of this phenomenon [10].

Recently, *E. equi* DNA was detected in a dog from North Carolina by use of species-specific ehrlichial primers [14]. As *E. equi* and the HGE agent are increasingly believed to be conspecific, this observation supports the position that HGE may occur in North Carolina. Additional studies are needed to further verify that infection with the HGE agent occurs in the Piedmont area of North Carolina, and, if this finding is verified, entomological studies to determine the vector should be undertaken.

Our findings have practical importance for clinicians. In our sample of patients, approximately one-half of those who sought care because of fever following a recent tick bite were diagnosed with a rickettsial or ehrlichial infection. These data justify the practice of using empiric therapy with doxycycline for all patients from areas endemic for rickettsial or ehrlichial disease who present with unexplained fever and a known or suspected recent tick bite. In addition, our data indicate that thrombocytopenia and low or low-to-normal leukocyte counts strongly suggest either ehrlichial or rickettsial infection in febrile patients with a history of recent tick bite.

Further prospective studies of patients with tick bite and fever from other endemic areas are needed to confirm and expand upon our findings. For example, it would be useful to know the incidence of ehrlichial and rickettsial infection in patients without a history of tick bite who are found to have fever and thrombocytopenia or leukopenia during the spring and summer months in endemic regions such as North Carolina.

References

1. Philip RN, Casper EA, Ormsbee RA, et al. Microimmunofluorescence test for the serological study of Rocky Mountain spotted fever and typhus. J Clin Microbiol **1976**;3:51–61.
2. Dawson JE, Rikshisa Y, Ewing SA, Fishbein DB. Serologic diagnosis of human ehrlichiosis using two *Echlichia canis* isolates. J Infect Dis **1991**; 163:564–67.
3. Igbal Z, Rikisisa Y. Isolation of *Echlichia canis* from blood and the tissue of dogs after doxycycline treatment. J Clin Microbiol **1994**;32:1644–49.
4. Wong SJ, Brady GS, Dumler JS. Serological responses to *Ehrlichia equi, Ehrlichia chaffeensis,* and *Borrelia burgdorferi* in New York State. J Clin Microbiol **1997**;35:2198–2205.
5. Asanovich KM, Bakken JS, Madigan JE, et al. Antigenic diversity of granulocytic ehrlichia species isolates from humans in Wisconsin, New York, and California horse. J Infect Dis **1997**; 176:1029–34.
6. Sexton, DJ, Corey GR, Carpenter C, et al. Dual infection with *Ehrlichia chaffeensis* and a spotted fever group rickettsia: a case report. Emerging Infect Dis **1998**;4:311–16.
7. Edelman DC, Dumler JS. Evaluation of an improved PCR diagnostic assay for human granulocytic ehrlichiosis. Molecular Diagnosis **1996**;1:41–49.
8. Chen S, Dumler JS, Bakken JS, Walker DH. Identification of a granulocytotropic ehrlichia species as the etiologic agent of human disease. J Clin Microbiol **1994**;32:589–95.
9. StataCorp. Stata Statistical Software: Release 6.0. College Station, TX: Stata Corporation, **1999**.
10. Magnarelli LA, Izdo JW, Anderson JF, et al. Human exposure to a granulocytic ehrlichia and other tick-borne agents in Connecticut. J Clin Microbiol **1998**;36:2823–27.
11. Wilfert CM, MacCormack JN, Kleeman K, et al. Epidemiology of Rocky Mountain spotted fever as determined by active surveillance. J Infect Dis **1984**;150:469–79.
12. Schubert JH. Serologic titers in rickettsial infection as affected by antibiotic treatment. Public Health Lab **1951**;10:38–41.
13. Bakken JS, Goellner P, Van Etten M, et al. Seroprevalence of human granulocytic ehrlichiosis among permanent residents of northwestern Wisconsin. Clin Infect Dis **1998**;27:1491–6.
14. Breitschwerdt EB, Hegarty BC, Hancock SI. Sequential evaluation of dogs naturally infected with *Ehrlichia canis, Ehrlichia chaffeensis, Ehrlichia equi, Ehrlichia ewingii* or *Bartonella vinsonii.* J Clin Microbiol **1998**;36:2645–51.