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

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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.
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 \textit{E. chaffeensis}, the HGE agent, and \textit{Rickettsia rickettsii} antigens by use of the indirect immunofluorescence antibody (IFA) test, as described elsewhere, except that \textit{E. chaffeensis} (Arkansas strain) was used in place of \textit{E. canis} [1–3]. Western blots were performed on selected sera with high titers against both \textit{ehrlichiae}. For Western blots, density gradient–purified \textit{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 \textit{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 \textit{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 \textit{nadA} gene and the 120-kDa protein gene of \textit{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 \textit{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.

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 dye-deoxyxynucleotide cycle sequencing method and an Applied Biosystems (Foster City, CA) model 377 automated fluorescent sequencer.

<table>
<thead>
<tr>
<th>Laboratory test</th>
<th>R. rickettsi* infection (n = 5)</th>
<th>\textit{E. chaffeensis} infection (n = 8)</th>
<th>\textit{E. chaffeensis} and HGE agent infection (n = 2)</th>
<th>Total (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;4-fold rise in R. rickettsi antibody titer</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>&gt;4-fold rise in \textit{E. chaffeensis} antibody titer or elevated acute antibody titer</td>
<td>0</td>
<td>8</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>&gt;4-fold rise in HGE agent antibody titer or elevated acute antibody titer</td>
<td>0</td>
<td>2*</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>PCR positive for \textit{E. chaffeensis}*</td>
<td>0/2</td>
<td>2/5*</td>
<td>1/1</td>
<td>3/21*</td>
</tr>
</tbody>
</table>

NOTE. HGE, human granulocytic ehrlichiosis; PCR, polymerase chain reaction; R. rickettsi, \textit{Rickettsia rickettsii}; \textit{E. chaffeensis}, \textit{Ehrlichia chaffeensis}.

* The patient with coinfection had negative serology but positive culture and tissue immunofluorescence assay for \textit{R. rickettsi}.

† Total includes 19 patients without evidence of ehrlichial or rickettsial infection.

‡ Both patients had \textit{E. chaffeensis} infection confirmed by other methods. Elevated titers to the HGE agent were felt to be cross-reacting antibodies to \textit{E. chaffeensis}.

§ Not all patients were tested. Fractions are number positive/number tested in each category.

† One additional patient was found to have \textit{E. chaffeensis} by PCR with eubacterial 16S rDNA primers and subsequent sequence analysis.

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 \textit{R. rickettsi} 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 \textit{R. rickettsi} antigens but had a positive rickettsial blood culture and a skin biopsy that showed organisms in arterioles that stained with immunofluorescent conjugates against \textit{R. rickettsi}, as well as evidence of \textit{E. chaffeensis} infection by serology and PCR.

A 4-fold or greater rise in \textit{E. chaffeensis} antibody titer was detected in 7 (20%) of 35 patients. Markedly elevated acute blood titers to \textit{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 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.

<table>
<thead>
<tr>
<th>Pathogen (n)</th>
<th>Mean WBC count × 10^9/L (SD)</th>
<th>Mean platelet cell count/μL (SD)</th>
<th>No. with rash (%)</th>
<th>No. hospitalized (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. rickettsii (5)</td>
<td>5.1 (3.7)^c</td>
<td>70,000 (53,000)^c</td>
<td>3 (60)</td>
<td>5 (100)^d</td>
</tr>
<tr>
<td>E. chaffeensis (8)</td>
<td>5.4 (4.7)^c</td>
<td>181,000 (165,000)^c</td>
<td>2 (25)</td>
<td>5 (63)^c</td>
</tr>
<tr>
<td>Confinfection with R. rickettsii and E. chaffeensis (1)</td>
<td>2.7</td>
<td>6900</td>
<td>1 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Agent of human granulocytic ehrlichiosis (2)</td>
<td>5.8 (4.4)</td>
<td>121,000 (68,000)</td>
<td>0 (0)</td>
<td>1 (50)</td>
</tr>
<tr>
<td>Patients without evidence of ehrlichial or rickettsial infection (19)</td>
<td>10.1 (4.3)^c</td>
<td>259,000 (72,000)^c</td>
<td>6 (32)</td>
<td>3 (16)</td>
</tr>
</tbody>
</table>

**NOTE.** The patient coinfeeted 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 range: 3.2–9.8 × 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 co-infection 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 seroprevalence 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