Expanded Geographic Distribution and Clinical Characteristics of Ehrlichia ewingii Infections, United States

Rebecca M. Harris, Brianne A. Couturier, Stephan C. Sample, Katrina S. Coulter, Kathleen K. Casey, Robert Schlaberg

Ehrlichiosis is a bacterial zoonosis, spread through the bites of infected ticks, that is most commonly caused in the United States by infection with the bacterium Ehrlichia chaffeensis. We retrospectively reviewed samples from an 18-month study of ehrlichiosis in the United States and found that E. ewingii was present in 10 (9.2%) of 109 case-patients with ehrlichiosis, a higher rate of infection with this species than had previously been reported. Two patients resided in New Jersey and Indiana, where cases have not been reported. All patients with available case histories recovered. Our study suggests a higher prevalence and wider geographic distribution of E. ewingii in the United States than previous reports have indicated.

Ehrlichiosis is prevalent in dogs and white-tailed deer throughout the central and southeastern United States, and reported human infections have increased since the disease became reportable in 2008. The first human cases of infections were reported in 4 patients in Missouri in 1999, three of whom were immunosuppressed (9). E. ewingii was subsequently reported in 4 symptomatic patients from Missouri, Oklahoma, and Tennessee, all of whom were co-infected with HIV (10). One case of E. ewingii infection that was likely acquired through platelet transfusion from an asymptomatic donor with tick exposure has been reported (11). Most recently, E. ewingii was detected in the peripheral blood and bone marrow of a symptomatic 65-year-old woman from Arkansas (12). The most consistent clinical findings in these patients were fever and thrombocytopenia. No deaths have been reported.

Ehrlichia spp. are obligate intracellular organisms, and morulae (bacterial clusters within cytoplasmic vacuoles) are visualized on peripheral blood films. However, detection of morulae in monocytes (for E. chaffeensis) or granulocytes (for E. ewingii) has limited sensitivity (13). Serologic testing for E. chaffeensis can be performed but is insensitive during the acute phase of illness and has limited specificity (5,9). Specific serologic testing for E. ewingii...
Methods

We retrospectively reviewed results of 18 months (May 2013–November 2014) of testing for human ehrlichiosis by real-time PCR. All samples submitted to Associated Regional and University Pathologists Laboratories (Salt Lake City, UT, USA) for *Ehrlichia* and *Anaplasma* species by real-time PCR were included in the analysis. PCR-positivity rates for *Ehrlichia* spp. were calculated on the basis of results for individual patients.

We used a real-time PCR for *Ehrlichia* and *Anaplasma* species that detects *E. chaffeensis*, *E. muris*–like pathogen, *E. ewingii*, and *E. canis* (without differentiating *E. ewingii* and *E. canis*) and *A. phagocytophilum*. In brief, nucleic acids extracted from whole blood specimens by using the Chemagic MSM I Automated Extraction Platform and the Chemagen Blood Extraction Kit (Perkin Elmer, Waltham, MA, USA) were amplified by using primers specific for 16S rRNA gene and *Ehrlichia* and *Anaplasma* species–specific probes for identification (Table 1). The reaction was prepared by using a 5× custom real-time Master Mix and 4 mmol/L MgCl₂ (Promega, Madison, WI, USA) with the following amplification parameters: 50.0°C for 2 min; denaturation at 95.0°C for 2 min; and 50 cycles at 95.0°C for 5 s, 56.0°C for 20 s, and 76.0°C for 20 s on the Rotor-Gene. Fluorescence acquisition was of infection with *E. ewingii*. Thus, diagnosis of infection with *E. ewingii* is reliant on species-specific molecular testing. The purpose of this study was to determine the geographic distribution and clinical characteristics of PCR-confirmed *E. ewingii* infections in the United States.

Table 1. Primers and probes used in real-time PCR for *Ehrlichia* and *Anaplasma* species, United States†

| Name     | Sequence, 5′→3′            | Concentration, nmol/L | Species detected              |
|----------|-----------------------------|------------------------|-------------------------------|
| Primer 1 | GCATTACTCACCAGCTTGCACCT     | 250                    | –                             |
| Primer 2 | CAAGCCTAACACATGCAAGTCGACGC | 1,000                  | –                             |
| Probe 1  | MGB-FAM-AGGTATATAAGCATTGTCCT-EDQ | 200                    | *E. chaffeensis*              |
| Probe 2  | MGB-(AP642)-AAGCTTATAAGGACATTGTTACATTT-CC-EDQ | 200 | *E. muris*–like agent         |
| Probe 3  | MGB-(AP953)-GGCTATAATAGTATTTCCGAAAGT-CC-EDQ | 200 | *E. canis*                    |
| Probe 4  | MGB-(AP593)-CTATTAGGATAGTATTTCCGAAAGT-CC-EDQ | 200 | *E. ewingii*                  |
| Probe 5  | MGB-(AP525)-AAGGAAATGATATTTCCGACATT-CC-EDQ | 200 | *A. phagocytophilum*          |

†Use of Super A, T, or G (Epoch Biosciences Inc., Bothell, WA, USA).
enzymes. Results of serologic testing for *E. chaffeensis* were negative. The patient received a 1-week course of doxycycline, and his symptoms resolved.

Case-patient 3 was a 73-year-old man from Indiana who had subjective fevers, mild left lower abdominal pain, myalgia, and malaise. The patient reported a tick bite on his abdomen 2–3 weeks before presentation. His medical history included type 1 diabetes, hypertension, and stroke. Results of a physical examination were unremarkable. He was afebrile at presentation but had leukopenia and thrombocytopenia. Results for patients tested for infection with *E. chaffeensis* by ELISA (total antibodies against *B. burgdorferi*) were negative. The patient received a 3-week course of doxycycline and showed clinical improvement; however, he died 1 month later of unrelated bacterial sepsis.

Case-patient 4 was a 74-year-old man from Arkansas who had malaise, arthralgia, fever, and nonbloody diarrhea in for 2 weeks in July 2014. The patient reported 2 tick bites 2 weeks before presentation. His medical history included diffuse large B-cell lymphoma (treated with gemcitabine), lung cancer (treated with a right lobectomy), chronic obstructive pulmonary disease that required supplemental oxygen, coronary artery disease, and hypothyroidism. At presentation, he had hypotension (79/46 mm Hg), tachycardia (142 beats/min), and a fever (temperature 100.1°F). Laboratory testing showed leukopenia, thrombocytopenia, and morulae in neutrophils on a peripheral blood smear. Results of serologic testing for *E. chaffeensis* were negative. The patient received a 3-week course of doxycycline and showed clinical improvement; however, he died 1 month later of unrelated bacterial sepsis.

Case-patient 5 was a 73-year-old man from Missouri who had headache, fever, and nausea in August 2014. The patient reported a tick bite ≈2 months before presentation. His medical history included bladder cancer (treated with immunotherapy), a melanoma on his right arm (treated with surgical excision), type 1 diabetes, and hypothyroidism. He had leukopenia, thrombocytopenia, and increased levels of liver enzymes. Blood cultures at presentation and serologic results for *E. chaffeensis* were negative.

### Table 2. Results for patients tested for infection with *Ehrlichia* and *Anaplasma* species by real-time PCR, United States

| State* | No. tested | *E. chaffeensis* | *E. canis*/ewingii | A. phagocytophilum |
|--------|------------|-----------------|-------------------|-------------------|
| Arkansas | 3 | 0 (0) | 1 (33.3) | 0 (0) |
| Colorado | 3 | 1 (33.3) | 0 (0) | 0 (0) |
| Connecticut | 23 | 0 (0) | 0 (0) | 3 (13) |
| Georgia | 7 | 1 (14.2) | 0 (0) | 0 (0) |
| Illinois | 70 | 4 (5.7) | 0 (0) | 0 (0) |
| Indiana | 263 | 25 (9.5) | 3 (1.1) | 0 (0) |
| Iowa | 4 | 1 (25) | 0 (0) | 0 (0) |
| Kansas | 52 | 10 (19.2) | 0 (0) | 0 (0) |
| Kentucky | 26 | 8 (30.8) | 0 (0) | 0 (0) |
| Louisiana | 11 | 1 (9.1) | 0 (0) | 0 (0) |
| Maine | 375 | 0 (0) | 0 (0) | 17 (4.5) |
| Massachusetts | 526 | 0 (0) | 0 (0) | 40 (7.6) |
| Michigan | 3 | 1 (33.3) | 0 (0) | 0 (0) |
| Minnesota | 930 | 0 (0) | 0 (0) | 44 (4.7) |
| Missouri | 94 | 15 (15.9) | 4 (4.3) | 0 (0) |
| Nebraska | 69 | 8 (11.6) | 0 (0) | 1 (1.4) |
| New Hampshire | 708 | 1 (0.1) | 0 (0) | 51 (7.2) |
| New Jersey | 202 | 7 (3.5) | 1 (0.5) | 5 (2.5) |
| New York | 283 | 3 (1.1) | 1 (0.4) | 6 (2.1) |
| Pennsylvania | 72 | 2 (2.8) | 0 (0) | 1 (1.4) |
| Tennessee | 150 | 6 (4) | 0 (0) | 0 (0) |
| Texas | 52 | 2 (3.8) | 0 (0) | 1 (1.9) |
| Utah | 16 | 1 (6.3) | 0 (0) | 0 (0) |
| Virginia | 28 | 0 (0) | 0 (0) | 1 (3.6) |
| Washington | 24 | 2 (8.3) | 0 (0) | 0 (0) |
| Wisconsin | 68 | 0 (0) | 0 (0) | 9 (13.2) |

*States for which all patients showed negative results are Alabama (n = 2), Arizona (n = 11), California (n = 19), Florida (n = 9), Hawaii (n = 1), Idaho (n = 1), Montana (n = 1), Nevada (n = 3), North Carolina (n = 6), North Dakota (n = 2), Ohio (n = 25), Oregon (n = 8), Rhode Island (n = 3), South Dakota (n = 21), and Wyoming (n = 3).

### Table 3. Associated laboratory findings for 5 patients infected with *Ehrlichia ewingii*, United States

| Laboratory finding | Patient | 1 | 2 | 3 | 4 | 5 |
|--------------------|---------|---|---|---|---|---|
| Minimum leukocyte count, × 10³ cells/μL | 3.5 | 4.5 | 2.7 | 2.6 | 2.8 |
| Minimum platelet count, × 10³/μL | 128 | 179 | 102 | 92 | 100 |
| Maximum aspartate aminotransferase level, U/L | 24 | 115 | 38 | 28 | 91 |
| Maximum alanine aminotransferase level, U/L | 26 | 279 | 38 | 28 | 78 |
| Maximum alkaline phosphatase level, U/L | 77 | 90 | 85 | 87 | 146 |
The patient received a 10-day course of doxycycline, and his symptoms resolved.

**Discussion**

In our study, *E. ewingii* accounted for 10 (9.2%) of 109 cases of *Ehrlichia* spp. infections, compared with only 31 (2.0%) of 1,518 reported human hantiviruses cases in the United States (4). Underreporting might be caused by successful empirical treatment without etiologic diagnosis; missed *E. ewingii* infections by serologic tests for *E. chaffeensis* in the acute phase of illness (case-patients 2, 4, and 5); and limited availability of molecular tests. Even with molecular testing, cases might be missed because of non-optimal timing of specimen collection and limited sensitivity of the assay, such as with testing after resolution of bacteremia.

Human infections with *E. ewingii* naturally acquired have been reported in Arkansas, Missouri, Oklahoma, and Tennessee (9,10,12). We now describe cases of infection in Indiana and New Jersey. The location of these cases is consistent with the known range of the vector (*A. americanum* ticks). *E. ewingii* has previously been reported in *A. americanum* ticks collected in New Jersey (14).

The 5 case-patients reported in this study had symptoms of classical ehrlichiosis, including fever, myalgia, malaise, and headache. Thrombocytopenia and leukopenia were the most consistent associated laboratory findings. All patients improved after treatment with doxycycline treatment, despite in some instances, major underlying disease. *E. ewingii* should be considered as an etiologic agent of tickborne febrile illness in the central and eastern United States that may be missed by serologic testing.

Dr. Harris is a clinical pathologist in The Mount Sinai Health System and at The Icahn School of Medicine at Mount Sinai, New York, New York. Her primary research interest is optimizing use of microbiological testing services.

**References**

1. Pritt BS, Sloan LM, Johnson DK, Munderloh UG, Paskewitz SM, McElroy KM, et al. Emergence of a new pathogenic *Ehrlichia* species, Wisconsin and Minnesota, 2009. N Engl J Med. 2011;365:422–9. http://dx.doi.org/10.1056/NEJMoa1010493

2. Chen SM, 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.

3. Johnson DK, Schifman EK, Davis JP, Neitzel DF, Sloan LM, Nicholson WL, et al. Human infection with *Ehrlichia muris*—like pathogen, United States, 2007–2013. Emerg Infect Dis. 2015;21:1794–9.

4. Adams D, Fullerton K, Jajosky R, Sharp P, Onweh D, Schley A, et al. Summary of notifiable infectious diseases and conditions—United States, 2013. MMWR Morb Mortal Wkly Rep. 2015;62:1–122. http://dx.doi.org/10.15585/mmwr.mm6253a1

5. Dumler JS, Madigan JE, Pusterla N, Bakken JS. Ehrlichioses in humans: epidemiology, clinical presentation, diagnosis, and treatment. Clin Infect Dis. 2007;45(Suppl 1):S45–51. http://dx.doi.org/10.1086/518146

6. Dahlgren FS, Mandel EJ, Krebs JW, Massung RF, McQuiston JH. Increasing incidence of *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* in the United States, 2000–2007. Am J Trop Med Hyg. 2011;85:124–31. http://dx.doi.org/10.4269/ajtmh.2011.10-0613

7. Beall MJ, Alleman AR, Breitschwerdt EB, Cohn LA, Couto CG, Dryden MW, et al. Seroprevalence of *Ehrlichia canis, Ehrlichia chaffeensis* and *Ehrlichia ewingii* in dogs in North America. Parasit Vectors. 2012;5:29. http://dx.doi.org/10.1186/1756-3305-5-29

8. Yabsley MJ, Varela AS, Tate CM, Dugan VG, Stallknecht DE, Little SE, et al. *Ehrlichia ewingii* infection in white-tailed deer (*Odocoileus virginianus*). Emerg Infect Dis. 2002;8:668–71. http://dx.doi.org/10.3201/eid0807.020018

9. Buller RS, Arens M, Hmiel SP, Paddock CD, Sumner JW, Rikhisa Y, et al. *Ehrlichia ewingii*, a newly recognized agent of human ehrlichiosis. N Engl J Med. 1999;341:148–55. http://dx.doi.org/10.1056/NEJM199907153410303

10. Paddock CD, Folk SM, Shore GM, Machado LJ, Huycke MM, Slater LN, et al. Infections with *Ehrlichia chaffeensis* and *Ehrlichia ewingii* in persons coinfected with human immunodeficiency virus. Clin Infect Dis. 2001;33:1586–94. http://dx.doi.org/10.1086/323981

11. Regan J, Matthias J, Green-Murphy A, Stanek D, Bertholf M, Pritt BS, et al. A confirmed *Ehrlichia ewingii* infection likely acquired through platelet transfusion. Clin Infect Dis. 2013;56:e105–7. http://dx.doi.org/10.1093/cid/cit177

12. Allen MB, Pritt BS, Sloan LM, Paddock CD, Musham CK, Ramos JM, et al. First reported case of *Ehrlichia ewingii* involving human bone marrow. J Clin Microbiol. 2014;52:4102–4. http://dx.doi.org/10.1128/JCM.01670-14

13. Hamilton KS, Standaert SM, Kinney MC. Characteristic peripheral blood findings in human ehrlichiosis. Mod Pathol. 2004;17:512–7. http://dx.doi.org/10.1038/modpathol.3800075

14. Schulze TL, Jordan RA, Schulze CJ, Mixson T, Papero M. Relative encounter frequencies and prevalence of selected Borrelia, *Ehrlichia*, and *Anaplasma* infections in *Amblyomma americanum* and *Ixodes scapularis* (Acari: Ixodidae) ticks from central New Jersey. J Med Entomol. 2005;42:450–6.

Address for correspondence: Robert Schlabel, Department of Pathology, University of Utah, 500 Chipeta Way, Salt Lake City, UT 84108, USA; email: robert.schlaberg@path.utah.edu