Detection of *Ehrlichia chaffeensis* DNA in Amblyomma americanum Ticks in Connecticut and Rhode Island

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*Ehrlichia chaffeensis*, the causative agent of human monocytic ehrlichiosis, is transmitted by *Amblyomma americanum* ticks, which are most abundant in the southern United States. Because serologic evidence suggests that residents of Connecticut are exposed to *E. chaffeensis*, *A. americanum* ticks were collected in Connecticut and Rhode Island for PCR analysis to detect *E. chaffeensis* DNA. Eight of 106 (7.5%) *A. americanum* ticks from Connecticut and 6 of 52 (11.5%) from Rhode Island contained *E. chaffeensis* DNA. Thus, *E. chaffeensis* is present in ticks in southern New England and transmission of *E. chaffeensis* may occur there.

*Ehrlichia chaffeensis*, the etiologic agent of human monocytic ehrlichiosis (HME), belongs to the *Ehrlichia canis* genogroup (4, 12). Because of the geographic distribution of its arthropod vector, *Amblyomma americanum*, HME is most frequently encountered in the southeastern United States, where this tick is most abundant (4, 13). By contrast, in southern New England and southern New York State, *Ixodes scapularis* ticks are also abundant and transmit the agent of human granulocytic ehrlichiosis (HGE). For this reason, most ehrlichiosis cases in southern New England and New York State are thought to be caused by the HGE agent, a member of the *Ehrlichia phagocytophila* genogroup, rather than *E. chaffeensis*. However, a recent study in Connecticut reported that approximately one-third of patients with serologic evidence of ehrlichiosis were found to have antibodies to *E. chaffeensis* (7). Although some of these patients could have acquired *E. chaffeensis* infection during travel to states with a known risk for *E. chaffeensis* exposure, it is conceivable that other residents may have contracted *E. chaffeensis* infection in Connecticut if bitten by *A. americanum* ticks there. In a separate study from New York State, some patients suspected of having ehrlichiosis showed seroreactivity against *E. chaffeensis* by indirect fluorescent antibody (IFA) staining methods (14). Because immunoblot assays could not detect antibodies to *E. chaffeensis* in these sera, it was thought that there was cross-reactivity in the IFA assay, rather than there being specific evidence of *E. chaffeensis* infections.

The geographic distribution of *A. americanum*, the lone star tick, has expanded northward into New York State, whereas prior to 1970, no *A. americanum* was reported in New York (11). These ticks have been found in Connecticut, albeit not in the same abundance as *I. scapularis*. By contrast, *A. americanum* is abundant on parts of Prudence Island, R.I. (10). Therefore, based on the serologic data indicating *E. chaffeensis* infections in humans, combined with the presence of *A. americanum*, we hypothesized that transmission of *E. chaffeensis* could occur in Connecticut and Rhode Island. As a first step in testing this hypothesis we used PCR amplification of DNA from *A. americanum* ticks, collected in Connecticut and Rhode Island, to search for *E. chaffeensis* DNA.

**Tick collection and PCR analysis.** Adult *A. americanum* ticks from Prudence Island, R.I., were collected from vegetation in the fall of 1992 and were stored in 70% ethanol. In 1996, 1997, and 1998, nearly all *A. americanum* ticks collected were submitted by residents living mainly in coastal communities in Fairfield and New Haven Counties, Conn. Some ticks were removed while actively feeding, and others were not engorged. DNA was extracted from ticks using the QiAmp tissue kit (Qiagen, Valencia, Calif.), following the manufacturer’s instructions. Briefly, the ticks were crushed in 100 μl of lysis buffer and incubated for 1 h at 55°C and 10 min at 70°C, and the mixture was then applied to a spin column for centrifugation. After removal of cellular debris and subsequent washings, purified DNA was eluted from the column in 200 μl of Tris (10 mM, pH 8.0) and stored at −20°C until PCR amplification was performed. For PCR amplification, the primers HE1 and HE3 were used to target a portion of the 16S rRNA gene of *E. chaffeensis* as described previously (2, 3). In addition, the primer sets 8F and 9B and EWI and HE3 were used to assess the presence of HGE DNA and *Ehrlichia ewingii* DNA (3, 8). As a template for the PCR amplification, 5 μl of tick-extracted DNA was used and the reaction conditions were as described previously (2). Six of 52 (11.5%) *A. americanum* ticks collected on Prudence Island, R.I., contained *E. chaffeensis* DNA (Fig. 1). Of 106 *A. americanum* ticks removed from persons from Fairfield County, Conn., 8 (7.6%) contained *E. chaffeensis* DNA. As a control, PCR with primers specific for the agent of HGE and *E. ewingii* did not produce any amplification product. Aliquots of the ethanol used as a preservative were included as controls during the DNA extraction and PCR methods to exclude cross-contamination of ticks during storage. The laboratory space was never used for DNA extraction and PCR of *E. chaffeensis* DNA prior to this study, reducing the risk of contamination of samples. After the PCR amplification, the products from all positive samples (using HE1 and HE3 primers) were purified from the agarose gel and then sequenced using a dye-terminator sequencing reaction (Perkin-Elmer Applied Biosystems, Foster City, Calif.) and analyzed in an automated DNA sequencer (model 377; Applied Biosystems). Sequences were aligned with known sequences from GenBank. All 14 sequences were identical to the published *E. chaffeensis* 16S ribosomal gene sequence (1). In addition, 50 *I. scapularis* ticks...
collected from Fairfield County and 63 I. scapularis nymphal ticks from Prudence Island served as controls and were also tested for the presence of E. chaffeensis DNA. No E. chaffeensis DNA was detected in these I. scapularis ticks. All control samples from the preserving ethanol were negative in the PCR.

Discussion. Using PCR amplification procedures, we have shown that DNA of E. chaffeensis was present in 7.6 and 11.5% of the A. americanum ticks collected from Connecticut and Rhode Island, respectively. Comparison of the infection rates did not show a statistically significant difference ($z = 0.522$ and $P = 0.602$, with a 95% confidence interval of $-0.136$ to 0.0565). Infection rates of ticks may vary based on location and time of collection of ticks. Indeed, ticks in Rhode Island and Connecticut were collected at different times, and different collection methods were employed.

Previously, results of seroanalyses for E. chaffeensis antibodies in humans clinically suspected of having ehrlichiosis revealed possible exposure to E. chaffeensis in Connecticut (7). In addition, antibodies to E. chaffeensis have been detected in sera from white-footed mice collected as early as 1983 (6). In a previous study, hemocytes of A. americanum ticks were found to contain rickettsia-like organisms, although PCR analysis at that time could not confirm the presence of E. chaffeensis DNA (9). Differences in methods and techniques may account for the variable results between that study and the present study. Based on the combination of this previous serologic evidence and the data reported here, we suggest that transmission of E. chaffeensis, the causative agent of HME, probably occurs in southern New England.

Since 1995, ehrlichiosis has been a reportable disease in Connecticut, and testing for HGE is available to any physician in Connecticut; the testing is supported in part by the Emerging Infections Program administered jointly by the Connecticut Department of Health and Yale School of Epidemiology and Public Health. The incidence of HGE has been found to be between 24 and 57 cases per 100,000 (5). By contrast, testing for E. chaffeensis is not routinely performed and the incidence of HME is not known in the northeastern United States. Future studies that involve laboratory testing for human ehrlichiosis may need to include testing for E. chaffeensis in addition to screening for the HGE agent in order to evaluate the frequency of E. chaffeensis in southern New England and New York State. The HGE agent has been detected in white-tailed deer blood samples from widely separated sites in Connecticut (8). These animals might be harboring both ehrlichial organisms in areas where A. americanum and I. scapularis coexist. Further work on detection of E. chaffeensis and the HGE agent in deer and smaller mammals in the northeastern United States is warranted and may help clarify the natural cycle of transmission of E. chaffeensis.

Nucleotide sequence accession number. The E. chaffeensis DNA sequence was submitted to GenBank under accession number AF305074.

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REFERENCES