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Prevalence of *Ehrlichia ewingii* in *Amblyomma americanum* in North Carolina

Ticks as vectors of *Ehrlichia* parasites have been the subject of study in North Carolina since 1993. Recently, *Ehrlichia ewingii*, which causes canine granulocytic ehrlichiosis, was documented as causing human illness in Missouri (3). Previously, the Lone Star tick, *Amblyomma americanum*, was confirmed as the vector of this parasite in dogs (2). Accordingly, in 1999, we focused our efforts on determining the presence and infection rate of *E. ewingii* in *A. americanum.* Field-collected ticks from all three geographical sections of the state were preserved in 95% ethanol. DNA was extracted from 2,970 ticks and subjected to nested PCR analysis (9). DNA from one adult and four pools of nymphs of *A. americanum* was positive when tested with *Ehrlichia* groESL primers but failed to amplify with *Ehrlichia chaffeensis*- or *Ehrlichia phagocytophila*-like (HGE)-specific primers. The primer set GE2b-GE3x was then used to amplify the 5′ portion of the 16S rRNA gene, giving a characteristic 569-bp product (6). These products were gel purified and sent to Commonwealth Biotechnologies, Inc. (Richmond, Va.), for sequence analysis. The sequences were most closely related to those of *E. ewingii* when compared to sequences of accession no. U96436 (5) and M73227 (1) deposited in GenBank. From one to six base pair differences over a 500+ base pair region were noted between our sequences and the GenBank sequences. After DNA from the five *E. ewingii*-positive ticks was sequenced, the primer pair EE1-HE3 was used to identify DNA from three additional *E. ewingii*-positive ticks. A total of eight *A. americanum* ticks or pools were positive for *E. ewingii*, while a total of nine were positive for *E. chaffeensis.* Unlike others (8), we did not find *E. ewingii*-positive organisms among 1,349 *Dermacentor variabilis* or 51 *Ixodes scapularis* ticks tested, even though amplifiable DNA was present.

Infection rates for *E. ewingii* were low in adult *A. americanum* ticks, 0.4% (1 of 245) in females and 0.9% (2 of 217) in males, and a pool-positive rate of 4.7% (5 of 106) was obtained when 1,308 nymphs in 106 pools were tested for *E. ewingii*. However, a minimum field infection rate for the nymphs was 0.4% (5 of 1,308 ticks). Positive *A. americanum* ticks originated in six widely separated piedmont and coastal plain counties (Table 1), showing that *E. ewingii* has a wide distribution in North Carolina. To date, clinical human infections of *E. ewingii* have been detected only in Missouri (3), possibly due to the lack of serological diagnostic techniques that differentiate *E. chaffeensis* from *E. ewingii*. Considering the high annual incidence rates of human monocytic ehrlichiosis reported for North Carolina (4, 7), the *E. ewingii* infections in dogs (5), the large populations of *A. americanum* in North Carolina, and the high levels of human contact with this tick, we suspect that some of those cases of human monocytic ehrlichiosis were caused by *E. ewingii.*

TABLE 1. *E. ewingii*-positive Lone Star ticks in North Carolina

Collection no.	Yr collected	Sex or stage	No. of specimens	Confirmation	County
W5-85	1995	Nymph	10	Nested PCR	Randolph
GDS11	1998	Female	1	Nested PCR	Gates
W9813	1998	Nymph	10	Sequencing	Stanly
W9815	1998	Nymph	10	Sequencing	Stanly
W9816	1998	Nymph	10	Sequencing	Stanly
BE1F	1998	Male	1	Sequencing	Chatham
BE176	1998	Nymph	1	Sequencing	Brunswick
BE48I	1998	Male	1	Nested PCR	Wake

REFERENCES

1. **Anderson, B. E., C. E. Greene, D. C. Jones, and J. E. Dawson.** 1992. *Ehrlichia ewingii* sp. nov., the etiologic agent of canine granulocytic ehrlichiosis. Int. J. Syst. Bacteriol. **42:**299–302.
2. **Anziani, O. S., S. A. Ewing, and R. W. Barker.** 1990. Experimental transmission of a granulocytic form of the tribe Ehrlichieae by *Dermacentor variabilis* and *Amblyomma americanum* to dogs. Am. J. Vet. Res. **51:**929–931.
3. **Buller, R. S., M. Arens, S. P. Hmiel, C. D. Paddock, J. W. Sumner, Y. Rikihisa, A. Unver, M. Gaudreault-Keene, F. A. Manian, A. M. Liddell, N. Schmulewitz, and G. A. Storch.** 1999. *Ehrlichia ewingii*, a newly recognized agent of human ehrlichiosis. N. Engl. J. Med. **341:**148–155.
4. **Carpenter, C. F., T. K. Gandhi, L. K. Kong, G. R. Corey, S.-M. Chen, D. H. Walker, J. S. Dumler, E. Breitschwerdt, B. Hegarty, and D. J. Sexton.** 1999. The incidence of erhlichial and rickettsial infection in patients with unexplained fever and recent history of tick bite in central North Carolina. J. Infect. Dis. **180:**900–903.
5. **Goldman, E. E., E. B. Breitschwerdt, C. B. Grindem, B. C. Hegarty, J. J. Walls, and J. S. Dumler.** 1998. Granulocytic ehrlichiosis in dogs from North Carolina and Virginia. J. Vet. Intern. Med. **12:**61–70.
6. **Massung, R. F., K. Slater, J. H. Owens, W. L. Nicholson, T. N. Mather, V. B. Solberg, and J. G. Olson.** 1998. Nested PCR assay for detection of granulocytic ehrlichiae. J. Clin. Microbiol. **36:**1090–1095.
7. **McQuiston, J. H., C. D. Paddock, R. C. Holman, and J. E. Childs.** 1999. The human ehrlichioses in the United States. Emerg. Infect. Dis. **5:**635–642.
8. **Murphy, G. L., S. A. Ewing, L. C. Whitworth, J. C. Fox, and A. A. Kocan.** 1998. A molecular and serologic survey of *Ehrlichia canis*, *E. chaffeensis*, and *E. ewingii* in dogs and ticks from Oklahoma. Vet. Parasitol. **79:**325–339.
9. **Nicholson, W. L., M. B. Castro, V. L. Kramer, J. W. Sumner, and J. E. Childs.** 1999. Dusky-footed wood rats (*Neotoma fuscipes*) and reservoirs of granulocytic ehrlichiae (*Rickettsiales: Ehrlichieae*) in northern California. J. Clin. Microbiol. **37:**3323–3327.

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