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Lack of Seroreactivity to *Ehrlichia chaffeensis* Among Rodent Populations

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ABSTRACT: A retrospective serosurvey for antibodies to *Ehrlichia chaffeensis* was conducted on eight species of wild rodents (*Mus musculus, Oryzomys palustris, Peromyscus leucopus, Rattus norvegicus, Reithrodontomys humulis, Sciurus carolinensis, Sciurus niger,* and *Sigmodon hispidus*) from the southeastern United States. Serum samples (n = 281) collected between 1973 and 1993 were evaluated using an indirect fluorescent antibody test. All samples, screened at a dilution of 1:32, were negative for antibodies to *E. chaffeensis*. Sixty-three percent of the rodents tested were from areas where *E. chaffeensis* has been confirmed or is strongly suspected to be endemic. These data suggest limited or no involvement of rodents in the epidemiology of *E. chaffeensis*.

Key words: *Ehrlichia chaffeensis*, serology, rodents, epidemiology, survey.

Human ehrlichiosis in the United States is caused by two species of the rickettsial genus *Ehrlichia*. Human monocytic ehrlichiosis (HME), caused by *Ehrlichia chaffeensis,* is found principally in the southeastern and southcentral United States (McDade, 1990). Human granulocytic ehrlichiosis (HGE), caused by an unnamed organism in the *E. phagocytophila/E. equi* genogroup, is found principally in the northeastern and northcentral United States (Goodman et al., 1996). Both organisms cause clinically similar syndromes and patients characteristically present with fever, headache, muscle aches, malaise, and nausea (Fishbein et al., 1994).

White-tailed deer (*Odocoileus virginianus*) have been implicated as the principal reservoir host for *E. chaffeensis* (Dawson et al., 1994; Lockhart et al., 1997b). Experimental inoculation studies indicate that some rodents (C3H/HeJ mice and white-footed mice (*Peromyscus leucopus*)) are susceptible to infection with *E. chaffeensis* but suggest they are relatively poor hosts (Telford and Dawson, 1996). Lone star ticks, *Amblyomma americanum,* have been implicated as the principal vector based on positive PCR results (Anderson et al., 1993) and experimental transstadial transmission of *E. chaffeensis* by *A. americanum* (Ewing et al., 1995).

The major reservoirs and vector for HGE are thought to be wild rodents and *Ixodes scapularis,* respectively (Walker and Dumler, 1996; Telford et al., 1996). Transmission of the HGE organism has been accomplished using field collected *I. scapularis* (Telford et al., 1996), and PCR analysis has revealed DNA corresponding to the HGE organism in *I. scapularis* from Wisconsin (USA) and Connecticut (USA) (Pancholi et al., 1995; Magnarelli et al., 1995).

Rodent models have been used to study the immunology and pathogenesis of ehrlichial infections of veterinary importance (Williams and Timoney, 1994), and currently rodent models of human ehrlichiosis are being evaluated. *Ehrlichia muris* (Wen et al., 1995), which is closely related to *E. chaffeensis,* was isolated from the spleen of a mouse (*Eothenomys kageus*) in Japan suggesting that wild rodents may be natural hosts for other ehrlichial agents. The purpose of this study was to retrospectively evaluate wild rodents for serologic evidence of infection with *E. chaffeensis* from the general endemic region.

The Southeastern Cooperative Wildlife Disease Study (SCWDS) (College of Veterinary Medicine, The University of Georgia, Athens, Georgia, USA) and Centers

TABLE 1. Species composition of 245 rodents examined for retrospective *E. chaffeensis* serology.

Species	N	Location	Map coordinates	Date
Cotton rat				
(*Sigmodon hispidus*)	8	Gwinnett Co., GA	33°54′N, 84°04′W	March 1980
	9	Clarke Co., GA	33°57′N, 83°23′W	October 1989
	74	Oglethorpe Co., GA	33°52′N, 83°06′W	March 1990
	29	Georgetown Co., SC	33°22′N, 79°17′W	December 1991
	2	Sebastian Co., AR	35°12′N, 94°15′W	April 1992
	11	Cumberland Co., NC	35°03′N, 78°52′W	February–July 1992
Total	133			
Gray squirrel				
(*Sciurus carolinensis*)	15	Chatham Co., GA	32°04′N, 81°05′W	May/June 1981
	32	Chatham Co., GA		October 1983
Total	47			
Fox squirrel				
(*Sciurus niger*)	10	Chatham Co., GA		May/June 1983
	10	Chatham Co., GA		October 1983
Total	20			
Norway rat				
(*Rattus norvegicus*)	16	Clarke Co., GA		August 1973
	4	Gwinnett/Banks Co., GA	34°20′N, 83°30′W	February/March 1980
Total	20			
House mouse				
(*Mus musculus*)	11	Gwinnett/Banks Co., GA		February/March 1980
	8	Clarke Co., GA		October 1989
	2	Cumberland Co., NC		June 1992
Total	19			
White-footed mouse				
(*Peromyscus leucopus*)	2	Gwinnett Co., GA		February/March 1980
	2	Clarke Co., GA		March 1989
	11	Oglethorpe Co., GA		March 1990
	4	Sebastian Co., AR		April 1992
	5	Cumberland Co., NC		February–June 1992
	1	Liberty Co., GA	31°51′N, 81°36′W	March 1993
Total	36			
Eastern harvest mouse				
(*Reithrodontomys humulis*)	2	Oglethorpe Co., GA		March 1991
Rice rat				
(*Oryzomys palustris*)	1	Georgetown Co., SC		December 1991
	1	Liberty Co., GA	31°44′N, 81°26′W	March 1993
Total	2			
Cotton mouse				
(*Peromyscus gossypinus*)	3	Camden Co., GA	30°50′N, 81°33′W	July 1988
Peromyscus sp.	1	Oglethorpe Co., GA		March 1990

for Disease Control and Prevention (Atlanta, Georgia, USA) have maintained rodent serum samples collected from various past studies. Rodents were collected using Sherman traps (H.B. Sherman Traps, Inc, Tallahassee, Florida, USA). Blood samples were collected via cardiac puncture, allowed to clot, and serum was harvested and stored at −20 C until used. Samples included in this study were collected from eight species of rodents in Arkansas (USA), Georgia (USA), North Carolina (USA) and South Carolina (USA) from 1973 through 1993. The identity, number, origin, and collection date for rodents tested are presented in Table 1.

Indirect fluorescent antibody (IFA) testing for *E. chaffeensis* was conducted as

previously described (Dawson et al., 1991). Antigen, consisting of *E. chaffeensis*-infected DH82 canine macrophage cells (American Type Culture Collection, Rockville, Maryland, USA), was spotted onto slides and used to screen serum samples at a dilution of 1:32 in 0.01 M phosphate buffered saline (pH 7.2). Antibody conjugates used in this study were obtained from Kirkegaard and Perry Laboratories (Gaithersburg, Maryland, USA) and included fluorescein isothiocyanate (FITC)-labeled goat anti-rat IgG used for squirrels (*Sciurus* spp.), cotton rats (*Sigmodon hispidus*), Norway rats (*Rattus norvegicus*), and rice rats (*Orozomys palustris*); FITC-labeled goat anti-mouse IgG used for house mice (*Mus musculus*) and eastern harvest mice (*Reithrodontomys humulis*); and FITC-labeled goat anti-*Peromyscus leucopus* for white-footed mice. All conjugates were used at a 1:50 dilution in phosphate-buffered saline (PBS) (pH 7.2).

Positive control serum for mice was obtained from a C3H/HeJ mouse experimentally inoculated with 1.9×10^6 *E. chaffeensis*-infected DH82 canine macrophage cells (J. M. Lockhart, unpubl. data). This serum was obtained on post-inoculation day (PID) 21 and had an antibody titer of 1:256 to *E. chaffeensis* using FITC-labeled goat anti-mouse IgG (1:50 dilution). Negative control serum for mice was from a C3H/HeJ mouse inoculated with 2.7×10^6 uninfected DH82 cells, which tested negative for antibodies to *E. chaffeensis* on PID 21.

Two hundred eighty-one rodent serum samples collected from 1973 to 1993 were evaluated by IFA (Table 1). All samples were negative at a 1:32 dilution for the presence of antibodies reactive to *E. chaffeensis*.

Species-specific secondary antibodies were not utilized in this serologic survey; however, conjugates prepared using the most closely related species that were commercially available were used. Although species-specific conjugates would have been optimal, past studies (Ishikura et al., 1992) have been successful in using generic secondary antibody conjugate for detection of *Rickettsia* sp. in different rodent species.

Of the rodents tested, 174 (62%) were from three counties in Georgia where *E. chaffeensis* is known or suspected to be endemic. Isolates of *E. chaffeensis* have been obtained from white-tailed deer in Clarke and Chatham counties (Lockhart et al., 1997b; D. E. Stallknecht, unpubl. data), and deer from Oglethorpe County had a high prevalence of *E. chaffeensis*-reactive antibodies (Lockhart et al., 1997b). *Amblyomma americanum*, a confirmed vector of *E. chaffeensis* (Ewing et al., 1995), is abundant in all three of these counties, and *E. chaffeensis* DNA has been demonstrated in adult *A. americanum* from Clarke County (Lockhart et al., 1997a).

The absence of seroreactivity to *E. chaffeensis* among rodents may be related to their status as hosts for different life stages of *A. americanum*. Studies of parasitism of rodents by *A. americanum* have indicated that larvae are commonly present, nymphs are occasionally present, but adults are very rarely found (Koch and Dunn, 1980; Lavender and Oliver, 1996). Because transovarial transmission is not known to occur among the ehrlichiae including *E. chaffeensis* (Groves et al., 1975; Ewing et al., 1995), wild rodents appear to be at low risk of exposure through parasitism by larval *A. americanum*. However, occasional exposure of wild rodents by infected nymphs is possible because transstadial transmission has been documented for *A. americanum* nymphs that were infected as larvae (Ewing et al., 1995). Experimental analysis indicated that splenectomy was necessary to efficiently infect *P. leucopus* with *E. chaffeensis* while red-backed voles (*Clethrionomys gapperi*) were refractory to infection, suggesting that these two species may not be efficient reservoirs of *E. chaffeensis* in nature (Telford and Dawson, 1996).

A previous serologic survey of wild

mammals at the Clarke County site also failed to detect *E. chaffeensis*-reactive antibodies in an additional 81 rodents (seven species) (Lockhart et al., 1997a). Combined, these serologic surveys suggest that wild rodents have limited, if any, involvement in the epidemiology of *E. chaffeensis*.

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