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DETECTION OF ANTIBODIES REACTIVE WITH *EHRLICHIA CHAFFEENSIS* IN THE RACCOON

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ABSTRACT: Antibodies reactive with *Ehrlichia chaffeensis* were detected in raccoon (*Procyon lotor*) serum samples by using an indirect immunofluorescence assay. Samples from 411 raccoons trapped in the southeastern United States from 1977 to 1999 were tested. Serologically reactive samples with reciprocal titers of ≥16 were detected from 83 raccoons (20%) from 13 of 16 counties in eight states, indicating that raccoons are commonly exposed to *E. chaffeensis*. Samples collected as early as 1977 were positive. A polymerase chain reaction assay specific for *E. chaffeensis* failed to detect the presence of ehrlichial DNA in serum samples from 20 representative seroreactive raccoons. Because of serologic cross-reactivity among antigens derived from different *Ehrlichia* spp., additional immunologic, molecular, or culture-based studies will be required to confirm *E. chaffeensis* infections of raccoons in the southeastern United States.

Key words: *Ehrlichia chaffeensis*, immunofluorescence assay, *Procyon lotor*, raccoon, survey.

INTRODUCTION

In the USA, human ehrlichiosis is caused by three distinct *Ehrlichia* spp.: *E. chaffeensis*, *E. ewingii*, and the human granulocytic ehrlichiosis agent (HGE, putatively *E. phagocytophila*). The clinical diseases caused by these three pathogens are similar, although evidence indicates that infection with *E. chaffeensis* may result in more severe disease (Standaert et al., 1998). The greatest number of cases reported have been due to infection with *E. chaffeensis*, which was first recognized in 1986 (Maeda et al., 1987; Eng et al., 1990; Anderson et al., 1991; Fishbein et al., 1994). As of 1997, there had been 742 cases of *E. chaffeensis* reported, and most of these cases occurred in the south-central and southeastern areas of the USA (McQuiston et al., 1999). Several studies suggest that the lone star tick, *Amblyomma americanum*, is the primary vector of *E. chaffeensis* (Anderson et al., 1993; Lockhart et al., 1995, 1996).

It is important to understand the natural history of *E. chaffeensis* so that measures for disease prevention may be designed and implemented; however, knowledge of the maintenance cycle of *E. chaffeensis* is incomplete. Isolation of *E. chaffeensis* from white-tailed deer (*Odocoileus virginianus*) (Lockhart et al., 1997b), molecular studies of tick vectors (Anderson et al., 1992; Anderson et al., 1993), and studies of experimental transmission between deer and *A. americanum* (Ewing et al., 1995) indicate that deer are important reservoir hosts of *E. chaffeensis* and that the agent may be maintained in a natural cycle involving white-tailed deer and lone star ticks.

Other wildlife species are suspected to play a role in the epizootiology of *E. chaffeensis*. Lockhart et al. (1997a) reported that nine of 43 raccoons (*Procyon lotor*) (21%) trapped at an *E. chaffeensis*-enzootic site in Georgia had antibodies reactive with *E. chaffeensis*. Because the raccoon is heavily parasitized by immature and adult *A. americanum* (Tugwell and Lancaster, 1962; Cooney and Burgdorfer, 1974; Koch and Dunn, 1980; Zimmerman et al., 1988), it is likely that raccoons are routinely exposed to this pathogen and may possibly be involved in its maintenance. We conducted a serosurvey using an indirect immunofluorescence assay (IFA) to evaluate the prevalence of antibodies reactive to *E. chaffeensis* among raccoons in the southeastern USA in order to better define the

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role of the raccoon in the natural history of this pathogen.

MATERIALS AND METHODS

Acquisition of samples and selection of controls

Archived raccoon serum samples were obtained through the Southeastern Cooperative Wildlife Disease Study (College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA). These samples were collected between 1977 and 1987 and had been stored at −20 C. Samples from Liberty and Wakulla Counties (Florida, USA) were collected in 1999 from areas in the general vicinity of where several cases of human infection with *E. chaffeensis* have occurred (Sumner et al., 1999). Samples from Pinellas Co. (Florida) were collected in 1998 as part of an oral rabies vaccine study.

A raccoon serum sample from Whatcom Co. (Washington, USA) was selected as a negative control because it gave no fluorescence in the IFA and was collected far outside the geographic range of *A. americanum* (Means and White, 1997). A raccoon serum sample empirically selected by screening samples from an area where *E. chaffeensis* is enzootic (Craven Co., North Carolina, USA) served as a positive control. The reciprocal plateau endpoint titer of the sample was high (512), and the pattern of specific fluorescence it gave in the IFA (density, distribution, and intensity) was consistent with that observed with seropositive humans proven to be infected with *E. chaffeensis* by other tests (e.g., bacterial isolation). Aliquots of both control serum samples were frozen to ensure consistency during future testing.

Antigen preparation and use of IFA

Teflon-templated glass slides were obtained from Erie Scientific Co. (Portsmouth, New Hampshire, USA). *Ehrlichia chaffeensis* (Arkansas strain) was grown in DH82 canine macrophage cells, and infected cells were applied to the slides as described (Nicholson et al., 1997). Briefly, approximately 50 μl of living cells in culture medium (approximately 3×10^6 cells per ml) was applied to each well of the antigen slide, and slides were incubated for 15 min at 37 C in a humidified plastic chamber. Culture medium containing any nonadherent cells was then removed by vacuum pipetting, and the slides were air dried, fixed in acetone for 15 min, and stored at −70 C until used.

The IFA was conducted using conditions and reagents described previously for the HGE agent (Comer et al., 1999a). The diluent was modified by incorporating a 10% suspension of freeze-disrupted normal, uninfected DH82 cells (approximately 3×10^6 cells/ml culture medium) and 2% normal heat-inactivated goat serum to reduce potential background staining. Because the expected range of antibody titers could not be predicted, raccoon serum samples were screened at a 1/16 dilution; no difficulty in reading slides due to nonspecific background staining was evident at this dilution. Samples that were reactive at the 1/16 dilution were then titrated to endpoint. Fluorescein isothiocyanate-labeled goat anti-raccoon IgG (heavy and light chain specific) conjugate (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Maryland, USA) was used at an optimal working dilution (1/100). Multiple dilutions of conjugate were tested against multiple dilutions of positive and negative controls to determine this dilution ("checkerboard titration"). Samples that were contaminated with bacterial or fungal growth were filtered (Spin-X 0.22 μm filter, Costar Corp., Cambridge, Massachusetts, USA) before dilution series were prepared.

All titers ≥16 were expressed as the greatest reciprocal dilution in which specific ehrlichial reactivity was observed. Titers were log-transformed (base 2) for analysis (Thrusfield, 1995), and geometric mean titers (GMT) were calculated where appropriate. Statistical analysis was conducted using EpiInfo Version 6.04b (Dean et al., 1994). A P-value of ≤0.05 was considered significant.

Polymerase chain reaction studies

We attempted to amplify *E. chaffeensis* DNA from 20 raccoon serum samples which were selected to be representative of all seroreactive samples tested. These samples originated from three barrier island and three inland sites in five states. DNA extracts were prepared from serum as previously described (Comer et al., 1999b) and evaluated by using a nested polymerase chain reaction (PCR) assay to test for the presence of the *E. chaffeensis* variable length PCR target (VLPT) (Sumner et al., 1999). DNA extracted from *E. chaffeensis*-infected DH82 cells and a water blank were included as positive and negative controls, respectively.

RESULTS

We tested 411 raccoons collected from 16 counties in eight states (Table 1, Fig. 1). The overall prevalence of reactivity with *E. chaffeensis* antigens was 20% (83 reactive raccoons from 13 counties in all states excluding Washington), but prevalence varied widely by county. Titers

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FIGURE 1. Distribution of raccoon collection sites for studies on ehrlichiae. Refer to TABLE 1 for information on individual sites.

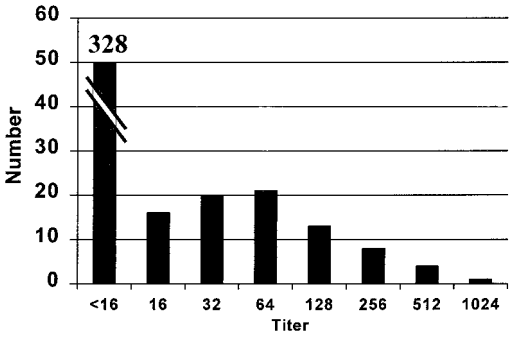


FIGURE 2. Frequency distribution of antibody titers to *Ehrlichia chaffeensis* in 411 raccoons.

ranged from 16 to 1,024, with a modal titer of 64 and an overall GMT of 60 (Fig. 2). Forty-six animals (11%) had a titer that exceeded the GMT for the group.

Higher prevalences of antibodies were significantly associated with raccoon samples from barrier islands when compared with maintained locations (χ^2 = 106.90, odds ratio = 15.70, confidence interval 8.23–30.17, $P < 0.001$). The prevalences of antibodies reactive with *E. chaffeensis* antigens of the three island populations sampled were 67% on Bull Island (South Carolina, USA), 80% on St. Catherines Island (Georgia, USA), and 88% on Ossabaw Island (Georgia).

Seropositive raccoons were detected each year in which raccoons were collected (Table 1). Seropositive raccoons were identified from several states in 1977 and 1978, the earliest years for which samples were available. The highest county-wide GMT observed (128) occurred in Craven County, North Carolina, and Brown County, Texas.

Markedly different prevalences were obtained from sites sampled from within a single state (Table 1). The very low prevalence in Okaloosa County (Florida) in 1979 (1%) was in marked contrast to seroprevalences among raccoons in closely situated counties in the Florida Panhandle in 1999 (Liberty Co., 75%; Wakulla Co., 91%). Pinellas County in central Florida, sampled in 1998, also had a low prevalence of reactive samples (<1%).

In the PCR assays, positive and negative DNA controls gave the expected results when the VLPT primers were used. However, no products were amplified from the raccoon serum samples. The samples ranged from 32 to 512 in antibody titer (GMT = 104) and came from populations where the prevalence ranged from 18% to 88%.

DISCUSSION

Overall, antibodies reactive with *E. chaffeensis* were detected from 20% of the raccoons sampled in this study. The prevalence of reactive antibodies on barrier islands was high (67%–88%) and significantly greater than the prevalence from mainland sites, although two inland coastal counties from the Florida Panhandle also had very high prevalences. Pung et al. (1994) speculated that *A. americanum* may be more abundant on barrier islands than on mainland sites when comparing the high tick burdens on raccoons from St. Catherines Island (Georgia) to those from other southeastern mainland counties. However, very high densities of *A. americanum* are common in many mainland regions, and this tick is a notorious pest species to humans and animals. Increased vector density should enhance *Ehrlichia* spp. transmission among vertebrate hosts, yet lower prevalences of *E. chaffeensis*-infected *A. americanum* were found on St.

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TABLE 1. Serologic reactivity of 414 raccoons to *Ehrlichia chaffeensis* antigens.

State	County	Map site	Location (map coordinates)	Year(s) collected	Number positive/ number tested[b]	Percent positive	Range of titers	GMT[a]
Florida	Glades	1	Fisheating Creek (26°59′N, 81°22′W)	1977	1/12	8	32	—
Florida	Liberty	2	Torreya State Park (30°17′N, 84°83′W)	1999	6/8	75	16–128	51
Florida	Okaloosa	3	Crestview (30°41′N, 86°59′W)	1979	1/88	1	16	—
Florida	Orange	4	unknown (28°34′N, 81°26′W)	1977	0/3	0	—	—
Florida	Pinellas	5	unknown (27°53′N, 82°72′W)	1998	2/160	1	16–64	32
Florida	Wakulla	6	Wakulla Springs Park (30°8′N, 84°39′W)	1999	10/11	91	16–1,024	104
Georgia	Chatham	7	Ossabaw Island (31°58′N, 81°12′W)	1981	21/34	88	16–256	46
Georgia	Liberty	8	St. Catherines Island (31°49′N, 81°48′W)	1977	8/10	80	16–128	54
North Carolina	Craven	9	Cherry Point (35°7′N, 77°05′W)	1979	5/11	45	32–512	128
North Carolina	Pender	10	unknown (34°30′N, 77°89′W)	1978	5/9	56	16–256	42
South Carolina	Barnwell	11	Savannah River Plant (33°17′N, 81°48′W)	unknown	0/1	0	—	—
South Carolina	Charleston	12	Bull Island (32°51′N, 79°86′W)	1987	16/24	67	16–512	73
Texas	Brown	13	Brownwood (31°46′N, 98°93′W)	1977, 1978	3/17	18	64–256	128
Virginia	James City	14	Williamsburg (37°18′N, 76°75′W)	1978	1/10	10	16	—
West Virginia	Ohio	15	unknown (40°6′N, 80°63′W)	1977	4/11	36	32–128	38
Washington	Whatcom	16	unknown (48°49′N, 121°76′W)	unknown	0/2	0	—	—
Total					83/411	20.2	16–1,024	60.4

[a] Geometric mean titers of ≥16.
[b] Positive titer ≥ 16.

Catherines Island (0.9%) and neighboring Sapelo Island (0.0%) than at a nearby mainland site (9.3%) (Whitlock et al., 2000). As a tick-borne disease, a focal distribution of ehrlichiosis would be expected, and differences in prevalence among collection sites presumably reflects the distribution of *E. chaffeensis* and the abundance of suitable tick vectors. The effect of vector density on transmission of *E. chaffeensis* is not fully understood.

A high antibody prevalence was noted in 1999 among raccoons from two counties in the Florida Panhandle from sites near locations where several human cases of ehrlichiosis due to *E. chaffeensis* have been reported (Paddock et al., 1997; Sumner et al., 1999). However, a low seroprevalence was detected in nearby Okaloosa County (using samples collected in 1979). This low prevalence may reflect a difference in the true prevalence of infection or a difference in the ecology of tick or reservoir host populations two decades ago (Lockhart et al., 1995).

Reactive samples were found in rac-

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coons collected in the late 1970s, indicating that *E. chaffeensis* may have been circulating among raccoons a decade before human infections with *E. chaffeensis* were initially recognized (Maeda et al., 1987). Furthermore, seroreactive raccoons were distributed in several southeastern states, indicating a widespread distribution of *E. chaffeensis.* Because they are readily fed upon by *A. americanum* and other ticks, are found throughout most of North America, and are easily trapped, raccoons may prove useful for surveillance of ehrlichiae and other tick-borne pathogens.

We used a minimum dilution of 1/16 in our screening assay of the raccoons. Other studies of *E. chaffeensis* infection in wildlife (Lockhart et al., 1997a,b; Little et al., 1998) have used 1/64 as a seropositive screening dilution. Because the spectrum of illness and magnitude of the antibody response associated with *E. chaffeensis* infection in raccoons is not known and could not be predicted, we reported the full range of titers obtained rather than assign an arbitrary cutoff value. Examination of Figure 2 reveals a normal distribution of log-transformed titers, a pattern well established for serologic data (Thrusfield, 1995). This evidence supports our approach. The distribution further suggests that a lower dilution might have been appropriate for screening our samples. Our assay has a strikingly low level of background staining and can be used to test lower dilutions of samples. Had a screening titer of 64 been used, the resulting frequency distribution would have been distinctly non-normal.

Serologic reactivity to *E. chaffeensis* antigens among the raccoons sampled does not preclude the possibility that some of the reactivity noted may have been caused by other, antigenically related, *Ehrlichia* spp. Potential for serologic cross-reaction also has been recognized for seropositive white-tailed deer (Dawson et al., 1996). Raccoons are host to immature and adult stages of at least three tick species which are recognized vectors of five *Ehrlichia* spp., although not all are known from the locations tested. Raccoons are heavily parasitized by both immature and adult *A. americanum* (Tugwell and Lancaster, 1962; Cooney and Burgdorfer, 1974; Koch and Dunn, 1980; Zimmerman et al., 1988), a putative vector of *E. chaffeensis* to deer and humans (Anderson et al., 1993; Lockhart et al., 1997b), *E. ewingii* to humans and dogs (Anziani et al., 1990; Buller et al., 1999), and the white-tailed deer *Ehrlichia* agent among deer (Dawson et al., 1996; Brandsma et al., 1999). Raccoons are also frequently parasitized by adult *Dermacentor variabilis* (Tugwell and Lancaster, 1962; Rabinowitz et al., 1983; Mock et al., 1991; Kollars, 1993; Kollars and Ladine, 1999), a tick species that has been incriminated as a secondary vector in the transmission of *E. canis* to dogs (Johnson et al., 1998). Furthermore, *E. chaffeensis* DNA was amplified from one of four *D. variabilis* removed from an opossum (*Didelphis virginiana*) in Arkansas, USA (Anderson et al., 1992). Raccoons are also important hosts for *Ixodes scapularis* larvae and nymphs (Fish and Daniels, 1990; Manelli et al., 1993), a vector of the HGE agent to animals and humans in the northeastern USA (Vignes and Fish, 1997). Multiple ehrlichial species have been reported from a single coastal island site in Georgia (Little et al., 1998). Thus, infection of raccoons by more than a single ehrlichial species is possible in certain locations where the pathogens and vector tick populations occur together.

Serologic cross-reactivity within the genus *Ehrlichia,* especially among species within the same genogroup (Dumler et al., 1995), has been useful in diagnostic testing. *Ehrlichia canis* was used as a surrogate antigen to detect human infection with *E. chaffeensis* before the latter organism was adapted to continuous cell culture (Eng et al., 1990). *Ehrlichia canis* and *E. chaffeensis* are presently used as surrogate antigens to detect *E. ewingii* infection in dogs and humans, respectively, because *E. ewingii* has not yet been propagated in

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continuous cell culture (Buller et al., 1999). Antibodies to the white-tailed deer agent (also not yet propagated in continuous cell culture) may cross-react with *E. chaffeensis* (Dawson et al., 1996), and at present only molecular methods are able to reliably detect the presence of this organism and *E. ewingii.* It is possible that some of the reactivity to *E. chaffeensis* antigens observed among raccoon serum samples in this study may represent reactivity to related species of *Ehrlichia.*

Further immunologic studies may provide more definitive confirmatory evidence that these raccoons were exposed to *E. chaffeensis.* Cultivation of the agent would be most desirable, and *E. chaffeensis* has been isolated from other animals, including white-tailed deer (Lockhart et al., 1997b) and goats (Dugan et al., 2000). However, other ehrlichial agents which potentially might have induced the antibody response observed (e.g., *E. ewingii* and the white-tailed deer *Ehrlichia* agent) have not yet been successfully isolated in cell culture. PCR amplification and sequencing might also be used to identify the etiologic agent. Adsorption with specific antigens or blocking with specific antibodies may help improve confirmatory assays (Comer et al., 1999c). Additional analysis of the immune response by Western immunoblotting methods would help to confirm the etiologic agent (Rikihisa et al., 1994).

Our inability to amplify ehrlichial DNA from serum specimens was not unexpected, as this substrate has yielded limited positive results when applied to acute-phase human serum samples (Comer et al., 1999b) or deer serum samples (Little et al., 1998). Furthermore, the stage of ehrlichial infection in the raccoons at the time of serum collection was unknown. More useful samples, such as EDTA-anticoagulated blood or splenic tissue, likely would have improved our molecular evaluation but were not available for testing. Experimental studies with captive raccoons would also be helpful in resolving the role that these hosts play in the maintenance of agents of human ehrlichiosis in nature and their transmission to humans. We hope that this serosurvey will stimulate additional research into these topics.

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LITERATURE CITED

ANDERSON, B., J. E. DAWSON, D. C. JONES, AND K. H. WILSON. 1991. *Ehrlichia chaffeensis,* a new species associated with human ehrlichiosis. Journal of Clinical Microbiology 29: 2838–2842.

———, K. G. SIMS, J. G. OLSON, J. E. CHILDS, J. F. PIESMAN, C. M. HAPP, G. O. MAUPIN, AND B. J. B. JOHNSON. 1993. *Amblyomma americanum*: a potential vector of human ehrlichiosis. American Journal of Tropical Medicine and Hygiene 49: 239–244.

———, J. SUMNER, J. DAWSON, T. TZIANABOS, C. GREENE, J. OLSON, D. FISHBEIN, M. OLSEN-RASMUSSEN, B. HOLLOWAY, E. GEORGE, AND A. AZAD. 1992. Detection of the agent of human ehrlichiosis by polymerase chain reaction. Journal of Clinical Microbiology 30: 775–780.

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. American Journal of Veterinary Research 51: 929–931.

BRANDSMA, A. R., S. E. LITTLE, J. M. LOCKHART, W. R. DAVIDSON, D. E. STALLKNECHT, AND J. E. DAWSON. 1999. Novel *Ehrlichia* organism (Rickettsiales: Ehrlichieae) in white-tailed deer associated with lone star tick (Acari: Ixodidae) parasitism. Journal of Medical Entomology 36: 190–194.

BULLER, R. S., M. ARENS, S. P. HIMEL, C. D. PADDOCK, J. W. SUMNER, Y. RIKIHISA, A. UNVER, M. GAUDREAULT-KEENER, F. A. MANIAN, A. M. LIDDELL, N. SCHMULEWITZ, AND G. A. STORCH. 1999. *Ehrlichia ewingii,* a newly recognized

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agent of human ehrlichiosis. New England Journal of Medicine 341: 148–155.

COMER, J. A., W. L. NICHOLSON, J. G. OLSON, AND J. E. CHILDS. 1999a. Serologic testing for human granulocytic ehrlichiosis at a national referral center. Journal of Clinical Microbiology 37: 558–564.

———, ———, J. W. SUMNER, J. G. OLSON, AND J. E. CHILDS. 1999b. Diagnosis of human ehrlichiosis by PCR assay of acute-phase serum. Journal of Clinical Microbiology 37: 31–34.

———, T. TZIANABOS, C. FLYNN, D. VLAHOV, AND J. E. CHILDS. 1999c. Serologic evidence of rickettsialpox (*Rickettsia akari*) infection among intravenous drug users in inner-city Baltimore, Maryland. American Journal of Tropical Medicine and Hygiene 60: 894–898.

COONEY, J. C., AND W. BURGDORFER. 1974. Zoonotic potential (Rocky Mountain spotted fever and tularemia) in the Tennessee Valley region. American Journal of Tropical Medicine and Hygiene 23: 99–108.

DAWSON, J. E., C. K. WARNER, V. BAKER, S. A. EWING, D. E. STALLKNECHT, W. R. DAVIDSON, A. A. KOCAN, J. M. LOCKHART, AND J. G. OLSON. 1996. *Ehrlichia*-like 16S DNA sequence from wild white-tailed deer (*Odocoileus virginianus*). The Journal of Parasitology 82: 52–58.

DEAN, A. G., J. A. DEAN, D. COULOUMBIER, K. A. BRENDEL, D. C. SMITH, A. H. BURTON, R. C. DICKER, K. SULLIVAN, R. F. FAGAN, AND T. G. ARNER. 1994. Epi-Info, Version 6.04b: A word processing, database and statistical program for epidemiology on microcomputers. Centers for Disease Control and Prevention, Atlanta, Georgia, 384 pp.

DUGAN, V. G., S. E. LITTLE, D. E. STALLKNECHT, AND A. D. BEALL. 2000. Natural infection of domestic goats with *Ehrlichia chaffeensis.* Journal of Clinical Microbiology 38: 448–449.

DUMLER, J. S., K. M. ASANOVICH, J. S. BAKKEN, P, RICHTER, R. KIMSEY, AND J. E. MADIGAN. 1995. Serologic cross-reactions among *Ehrlichia equi, Ehrlichia phagocytophila,* and human granulocytic ehrlichia. Journal of Clinical Microbiology 33: 1098–1103.

ENG, T. R., J. R. HARKNESS, D. B. FISHBEIN, J. E. DAWSON, C. N. GREEN, M. A. REDUS, AND F. T. SATALOWICH. 1990. Epidemiologic, clinical, and laboratory findings of human ehrlichiosis in the United States, 1988. Journal of the American Medical Association 264: 2251–2258.

EWING, S. A., J. E. DAWSON, A. A. KOCAN, R. W. BARKER, C. K. WARNER, R. J. PANCIERA, J. C. FOX, K. M. KOCAN, AND E. F. BLOUIN. 1995. Experimental transmission of *Ehrlichia chaffeensis* (Rickettsiales: Ehrlichieae) among white-tailed deer by *Amblyomma americanum* (Acari: Ixodidae). Journal of Medical Entomology 32: 368–374.

FISH, D., AND T. J. DANIELS. 1990. The role of medium-sized mammals as reservoirs of *Borrelia burgdorferi* in southern New York. Journal of Wildlife Diseases 26: 339–345.

FISHBEIN, D. B., J. E. DAWSON, AND L. E. ROBINSON. 1994. Human ehrlichiosis in the United States, 1985–1990. Annals of Internal Medicine 120: 736–743.

JOHNSON, E. M., S. A. EWING, R. W. BARKER, J. C. FOX, D. W. CROW, AND K. M. KOCAN. 1998. Experimental transmission of *Ehrlichia canis* (Rickettsiales: Ehrlichieae) by *Dermacentor variabilis* (Acari: Ixodidae). Veterinary Parasitology 31: 277–288.

KOCH, H. C., AND J. E. DUNN. 1980. Ticks collected from small and medium-sized wildlife hosts in LeFlore County, Oklahoma. Southwestern Entomologist 5: 214–221.

KOLLARS, T. M., JR. 1993. Ticks (Acari: Ixodidae) infesting medium-sized wild mammals in southwestern Tennessee. Journal of Medical Entomology 30: 896–900.

———, AND T. A. LADINE. 1999. Patterns of infestation by adult *Dermacentor variabilis* (Acari: Ixodidae) in a mark-recapture study of raccoons (Mammalia: Carnivora) and Virginia opossums (Mammalia: Didelphimorphia) in Tennessee. Journal of Medical Entomology 36: 263–267.

LITTLE, S. E., D. E. STALLKNECHT, J. M. LOCKHART, J. E. DAWSON, AND W. R. DAVIDSON. 1998. Natural coinfection of a white-tailed deer (*Odocoileus virginianus*) population with three *Ehrlichia* species. The Journal of Parasitology 84: 897–901.

LOCKHART, J. M., W. R. DAVIDSON, J. E. DAWSON, AND D. E. STALLKNECHT. 1995. Temporal association of *Amblyomma americanum* with the presence of *Ehrlichia chaffeensis* antibodies in white-tailed deer. Journal of Wildlife Diseases 31: 119–124.

———, ———, D. E. STALLKNECHT, J. E. DAWSON, AND S. E. LITTLE. 1997a. Natural history of *Ehrlichia chaffeensis* (Rickettsiales: Ehrlichieae) in the Piedmont physiographic region of Georgia. The Journal of Parasitology 83: 887–894.

———, ———, ———, AND ———. 1996. Site-specific geographic association between *Amblyomma americanum* (Acari: Ixodidae) infestations and *Ehrlichia chaffeensis*-reactive (Rickettsiales: Ehrlichieae) antibodies in white-tailed deer. Journal of Medical Entomology 33: 153–158.

———, ———, ———, ———, AND E. W. HOWERTH. 1997b. Isolation of *Ehrlichia chaffeensis* from white-tailed deer (*Odocoileus virginianus*) confirms their role as natural reservoir hosts. Journal of Clinical Microbiology 35: 1681–1686.

MAEDA, K., N. MARKOWITZ, R. C. HAWLEY, M. RISTIC, D. COX, AND J. E. MCDADE. 1987. Human infection with *Ehrlichia canis,* a leukocytic rick-

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ettsia. New England Journal of Medicine 316: 853–856.

MANELLI, A., U. KITRON, C. J. JONES, AND T. J. SLAJCHERT. 1993. *Ixodes* infestation on medium-sized mammals and blue jays in northwestern Illinois. Journal of Medical Entomology 30: 950–952.

MCQUISTON, J. H., C. D. PADDOCK, R. C. HOLMAN, AND J. E. CHILDS. 1999. The human ehrlichioses in the United States. Emerging Infectious Diseases 5: 635–642.

MEANS, R. G., AND D. J. WHITE. 1997. New distribution records of *Amblyomma americanum* (L.) (Acari: Ixodidae) in New York state. Journal of Vector Ecology 22: 133–145.

MOCK, D. E., D. B. BRILLHART, L. B. FOX, AND S. J. UPTON. 1991. Ticks (Acari: Ixodidae) from raccoons (*Procyon lotor*) in eastern Kansas, with a new state record for *Ixodes texanus.* Journal of the Kansas Entomological Society 64: 344–345.

NICHOLSON, W. L., J. A. COMER, J. W. SUMNER, C. GINGRICH-BAKER, R. T. COUGHLIN, L. A. MAGNARELLI, J. G. OLSON, AND J. E. CHILDS. 1997. An indirect immunofluorescence assay using a cell culture-derived antigen for detection of antibodies to the agent of human granulocytic ehrlichiosis. Journal of Clinical Microbiology 35: 1510–1516.

PADDOCK, C. D., J. W. SUMNER, G. M. SHORE, D. C. BARTLEY, R. C. ELIE, J. G. MCQUADE, C. R. MARTIN, C. S. GOLDSMITH, AND J. E. CHILDS. 1997. Isolation and characterization of *Ehrlichia chaffeensis* strains from patients with fatal ehrlichiosis. Journal of Clinical Microbiology 35: 2496–2502.

PUNG, O. J., L. A. DURDEN, C. W. BANKS, AND D. N. JONES. 1994. Ectoparasites of opossums and raccoons in southeastern Georgia. Journal of Medical Entomology 31: 915–919.

RABINOWITZ, A., S. PATTON, AND V. MAJOR. 1983. Ticks from raccoons of Cades Cove, Great Smokey Mountains National Park. Journal of the Tennessee Academy of Science 58: 29–30.

RIKIHISA, Y., EWING, S. A., AND J. C. FOX. 1994. Western immunoblot analysis of *Ehrlichia chaffeensis, E. canis,* or *E. ewingii* infections in dogs and humans. Journal of Clinical Microbiology 32: 2107–2112.

STANDAERT, S. M., L. A. CLOUGH, W. SCHAFFNER, J. S. ADAMS, AND K. M. NEUZIL. 1998. Neurologic manifestations of human monocytic ehrlichiosis. Infectious Diseases in Clinical Practice 7: 358–362.

SUMNER, J. W., J. E. CHILDS, AND C. D. PADDOCK. 1999. Molecular cloning and characterization of the *Ehrlichia chaffeensis* variable-length PCR target: an antigen-expressing gene that exhibits interstrain variation. Journal of Clinical Microbiology 37: 1447–1453.

THRUSHFIELD, M. 1995. Veterinary epidemiology, 2nd Edition. Blackwell Science Ltd., Malden, Massachusetts, 483 pp.

TUGWELL, P., AND J. L. LANCASTER, JR. 1962. Results of a tick-host study in northwest Arkansas. Journal of the Kansas Entomological Society 35: 202–211.

VIGNES, F., AND D. FISH. 1997. Transmission of the agent of human granulocytic ehrlichiosis by host-seeking *Ixodes scapularis* (Acari: Ixodidae) in southern New York state. Journal of Medical Entomology 34: 379–382.

WHITLOCK, J. E., Q. Q. FANG, L. A. DURDEN, AND J. H. OLIVER. 2000. Prevalence of *Ehrlichia chaffeensis* (Rickettsiales: Rickettsiaceae) in *Amblyomma americanum* (Acari: Ixodidae) from the Georgia coast and barrier islands. Journal of Medical Entomology 37: 276–280.

ZIMMERMAN, R. H., G. R. MCWHERTER, AND S. R. BLOEMER. 1988. Medium-sized mammal hosts of *Amblyomma americanum* and *Dermacentor variabilis* (Acari: Ixodidae) at Land Between the Lakes, Tennessee, and effects of integrated tick management on host infestations. Journal of Medical Entomology 25: 461–466.

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