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WHITE-TAILED DEER AS A POTENTIAL RESERVOIR OF *EHRLICHIA* SPP.

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ABSTRACT: We determined the antibody prevalence to *Ehrlichia* spp., in white-tailed deer (*Odocoileus virginianus*) and the geographic distribution of seropositive animals in 84 counties in Alabama, Arkansas, Florida, Georgia, Illinois, Kentucky, Louisiana, Maryland, Massachusetts, Mississippi, Missouri, North Carolina, South Carolina, Tennessee, Texas, Virginia, and West Virginia (USA). Using an indirect fluorescent antibody test we detected antibodies (≥1:128) to this bacterium in 544 (43%) of 1269 deer. Presence of antibodies to *Ehrlichia* spp. was related to a southerly latitude, low elevation, and resulting milder climatic conditions. It appears that white-tailed deer were naturally infected with *Ehrlichia* spp.; the infection was widely distributed throughout the southeastern United States. Based on these data, we propose that white-tailed deer play a role in the natural history of *Ehrlichia* spp. infection in the United States.

Key words: Human ehrlichiosis, *Ehrlichia* spp., white-tailed deer, wildlife reservoir, ticks, *Amblyomma americanum*, surveillance, serologic survey.

INTRODUCTION

Human ehrlichiosis in the United States was described by Maeda et al. (1987) and the etiologic agent, *Ehrlichia chaffeensis*, was isolated in 1990 (Dawson et al., 1991). The disease is characterized by a wide spectrum of clinical effects ranging from asymptomatic to fatal (Eng et al., 1990). The most common symptoms are fever, headache, malaise, myalgia, and nausea or vomiting, and, on occasion, rash (Fishbein and Dawson, 1990). Thrombocytopenia, leukopenia, and abnormal liver function test results frequently are noted. This disease is associated with a history of tick exposure (Fishbein et al., 1989). However, the vector or vectors of *E. chaffeensis* have not been identified with certainty.

Human cases of ehrlichiosis have been reported from 27 states in widely separated regions of the United States; most cases occur in the southeast and midwest (Fishbein and Dawson, 1990). Because human ehrlichiosis is not reportable through a national surveillance program, numbers of documented cases are relatively low. However, ehrlichiosis can be as prevalent as Rocky Mountain spotted fever, a reportable disease (Fishbein et al., 1989). The highest prevalence of human ehrlichiosis occurs in the spring and early summer, concurrent with the time of greatest host-seeking activity for adults and nymphs of several tick species (Bishopp and Trembley, 1945). Our objective was to determine the prevalence of *Ehrlichia* spp. in white-tailed deer of the southeastern U.S.

MATERIALS AND METHODS

Twelve hundred and sixty-nine white-tailed deer (*Odocoileus virginianus*) sera were collected from 84 counties in Alabama, Arkansas, Florida, Georgia, Illinois, Kentucky, Louisiana, Maryland, Massachusetts, Mississippi, Missouri, North Carolina, South Carolina, Tennessee, Texas, Virginia, and West Virginia (USA) from 1986 to 1993 (25°55′ to 42°40′N, 69°58′ to 104°50′W) (Fig. 1). Samples were obtained by wildlife agency personnel from hunter-killed male and female white-tailed deer. All sera were either tested fresh or stored at −20 C until analyzed.

Indirect immunofluorescence was used to detect immunoglobulins to *Ehrlichia* spp. with *Ehrlichia chaffeensis* that was acetone fixed to

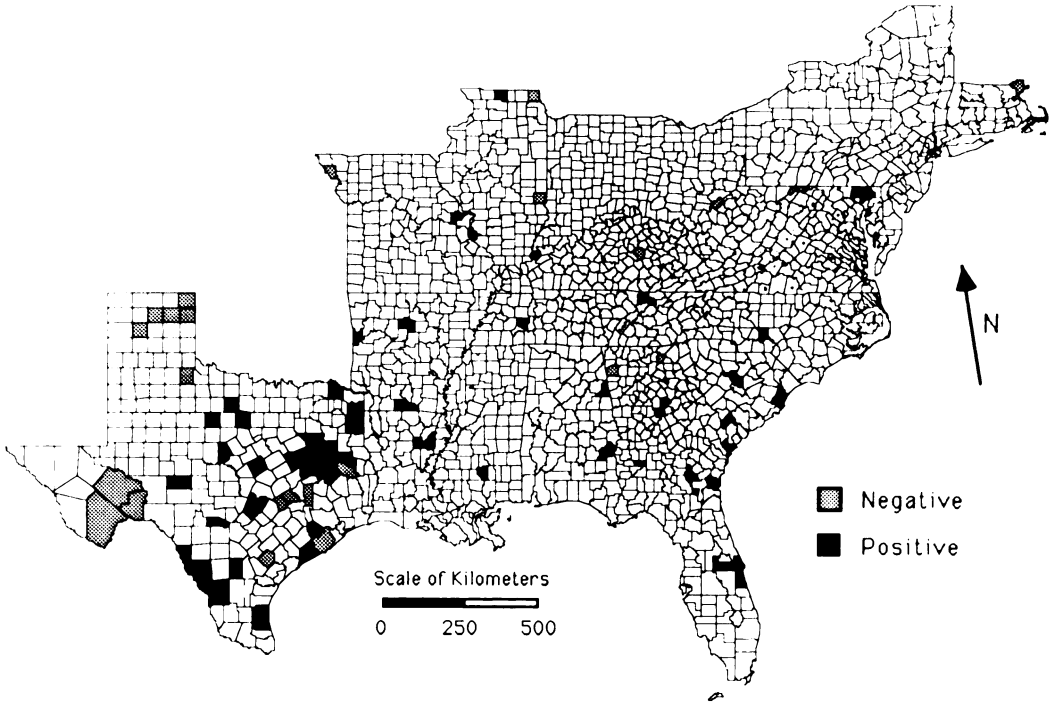

FIGURE 1. Partial map of the United States with the 84 counties where white-tailed deer sera were collected from 1986 to 1993. Counties with only seronegative deer are indicated by the light shading. The counties in black had at least one seropositive deer.

glass slides (Dawson et al., 1991). End-point titration of sera established the highest dilution at which fluorescence still could be detected using fluorescein isothiocyanate-labelled rabbit anti-deer immunoglobulin G (Kirkegaard & Perry Laboratories, Gaithersburg, Maryland) diluted 1:100 in phosphate-buffered saline (PBS). Positive control sera for white-tailed deer were selected from specimens whose reaction patterns with *E. chaffeensis* were identical to reaction patterns of known infected humans. Presumptive negative control sera were from white-tailed deer in Illinois where human ehrlichiosis is rare. Geometric mean titers (GMTs) were computed on positive samples (≥ 128).

We obtained 12 summary climatic or environmental variables from each county where deer were surveyed using a major city at the approximate center of the county as the reference point. These data included elevation, evaporation, longitude, latitude, precipitation, mean air temperature, mean soil temperature (estimated from mean air temperature), mean soil temperature in the summer, mean soil temperature in the winter, and three derived stress variables; moisture stress severity index (MSSI), temperature stress severity index (TSSI), and a summary climate stress severity index (CSSI). The stress variables were developed at the Soil Conservation Service (U.S. Department of Agriculture, Washington, D.C., USA) and are available upon request (H. Eswaran, P. Reich, pers. comm.). Indices varied from 0 to 1, with 1 indicating maximum climatic stress. Climatic values were means of 30 yr of observation, dating from 1951 to 1980.

Univariate comparisons of each climatic and environmental variable corresponding to counties grouped as seropositive (containing at least one deer with antibody to *Ehrlichia spp.*) or seronegative (containing no seropositive deer, but at least seven deer without positive antibody titers) were made using parametric (t-test for equal or unequal variances, as appropriate) or nonparametric (Mann-Whitney U-test) statistical tests (Sokal and Rohlf, 1981). The distribution of each variable according to the serologic grouping of the county was first examined for skewness before univariate tests were applied. To reduce the likelihood of misclassifying a county as seronegative, only counties with a minimum sample number of seven deer testing negative were included in this group. This permitted a high degree of certainty (>99%) that a positive deer would be detected based on the overall antibody prevalence of 43% (presuming

TABLE 1. Prevalence of antibodies to *Ehrlichia* spp. among white-tailed deer in the eastern United States, 1986 to 1993.

State	Sampling years	Number of counties	Number of samples	Number (%) positive	Geometric mean[a] titer	Range of antibody titer
Alabama	1991 and 1992	2	27	19 (70)	198	128–512
Arkansas	1991	3	75	40 (53)	284	128–1,024
Florida	1989, 1991	2	36	31 (86)	345	128–2,048
Georgia	1989 to 1991	9	301	195 (65)	369	128–4,096
Illinois	1989, 1993	4	205	5 (2)	169	128–256
Kentucky	1991	2	41	11 (26)	309	256–1,024
Louisiana	1992	2	11	9 (81)	203	128–512
Massachusetts	1986 to 1988	2	35	0	64	
Maryland	1989, 1991	3	145	82 (56)	422	128–8,192
Mississippi	1991	1	5	5 (100)	891	512–1,024
Missouri	1992	2	44	14 (31)	378	128–2,048
North Carolina	1991	1	22	13 (59)	414	128–2,048
South Carolina	1988, 1991 to 1992	3	26	8 (30)	279	128–1,024
Tennessee	1992	2	21	10 (47)	239	128–1,024
Texas	1992	44	232	71 (30)	223	128–2,048
Virginia	1992	1	31	31 (100)	876	128–8,192
West Virginia	1991	1	12	0	64	
Total	1986 to 1993	84	1,269	544 (43)	372	128–8,192

[a] A value of 64, average reciprocal titer for negative sera, was used for each negative sample in analyses.

the probability of a deer being seronegative = 0.47) (Sokal and Rohlf, 1981).

Discriminant analysis (procedure DISCRIMINANT; Norušis, 1990) was used to identify groups of variables important for distinguishing between counties with and without seropositive deer. Although discriminant analysis is considered a robust technique that can tolerate deviations from test assumptions, we examined the appropriateness of this application. A test for equality of group covariance matrices indicated no significant difference (Box's M = 29.7, $P > 0.05$) (procedure DISCRIMINANT; Norušis, 1990), but no tests were conducted concerning the multivariate normal distribution of discriminating variables. All variables meeting inclusion criterion ($F \geq 1.0$) were initially entered into the model and a backward elimination process minimizing Wilks' lambda was used to systematically delete variables (Klecka, 1980). This combination of forward and backward stepwise selection removed previously selected variables made redundant by the inclusion of variables or combinations of variables in subsequent steps. The probability of group membership (a county with or without seropositive deer) was set at two different levels in alternative discriminant analyses to account for the characteristics of the study variables. In the first analysis the prior probability of group selection was set at 0.5 to reflect the overall seroprevalence of *Ehrlichia* spp. antibody in deer (43%). In a second model 0.19 was selected as prior probability for inclusion in the seronegative county group and 0.81 was selected for inclusion in the seropositive county group. These probabilities reflected the numbers of counties actually containing seronegative ($n = 13$) and seropositive ($n = 54$) deer.

RESULTS

Five hundred and forty-four (43%) of the 1269 samples had antibody titers ≥1:128 to *E. chaffeensis*, or a closely related ehrlichial species (Table 1). Seropositive deer were found in 15 of 17 states examined. Antibody prevalence, however, varied greatly between locations within states (Fig. 1). In counties with seropositive deer, antibody prevalence ranged from 4.2% to 100%. Massachusetts was the only state with no seropositive deer; 35 deer were tested from Barnstable and Essex Counties.

The modal antibody titer for all positive deer was 1:256 with a maximum of 1:8,192. The overall GMT for titers ≥128 was 372. The five counties with the highest GMTs were Marion County, Mississippi ($\bar{x} = 891$; range = 512 to 1,024; $n = 5$); St. Charles County, Missouri ($\bar{x} = 883$; range = 128 to 2,048; $n = 14$); York County, Virginia ($\bar{x} = 876$; range = 128 to 8,192; $n = 31$); Jones

TABLE 2. Comparisons of climatic and environmental variables associated with counties from which deer were found to be seropositive or seronegative for *Ehrlichia* spp. antibodies. Only counties with a minimum of seven negative deer were included in the seronegative total.

Variable	Seropositive counties (n = 59)		Seronegative counties (n = 14)	
	Mean[a]/median[b]	Range	Mean[a]/median[b]	Range
Number of deer tested per county	16[b]	1–109	25[b]	7–66
Elevation (m)[c]	149 ± 129[a]	3–652	360 ± 288[a]	8–914
Evaporation (mm)[d]	962 ± 125[a]	662–1,264	792 ± 124[a]	625–1,052
Longitude (W)	92[b]	76–100	88[b]	70–102
Latitude (N)[e]	32[b]	27–42	37[b]	28–42
Precipitation (mm)	1,061 ± 257[a]	512–1,528	966 ± 365[a]	310–1,556
Air temperature (C)	18 ± 3[a]	8–23	14 ± 3[a]	8–20
Soil temperature (C)[d]	20 ± 3[a]	11–26	16 ± 3[a]	11–23
Soil temperature (summer) (C)[d]	26 ± 2[a]	20–30	23 ± 3[a]	18–27
Soil temperature (winter) (C)[d]	14 ± 4[a]	2–21	9 ± 4[a]	2–18
Moisture Stress Severity Index[f]	0.13[b]	0–0.89	0.15[b]	0.0–0.98
Temp Stress Severity Index[g,c]	0.06[b]	0.0–0.42	0.23[b]	0.0–0.42
Climate Stress Severity Index[g,h]	0.19[b]	0.0–0.90	0.38[b]	0.0–0.98

[a] Mean ± SD.
[b] Median values.
[c] $P \leq 0.05$, t-test for equal or unequal variances.
[d] $P \leq 0.01$, t-test for equal or unequal variances.
[e] $P \leq 0.05$, Mann-Whitney U-test.
[f] Expression of the intensity of lack of water for plant growth and is calculated as: part of the year that the soil is completely dry plus part of the year that the soil is partly dry. Partly dry is defined as having a soil water tension >33 kilopascals (kPa); completely dry is defined as having a water tension >1,500 kPa.
[g] Expresses temperature stress and is calculated as: part of the year that the soil temperature is below 5 C.
[h] Calculated as the sum of moisture and temperature stress indices.

County, Georgia ($\bar{x}$ = 545; range = 256 to 1,024; n = 11); and Union County, Arkansas ($\bar{x}$ = 464; range = 256 to 1,024; n = 7).

Prevalence of seropositive deer was highly variable among counties in each state (Fig. 1). In general, counties with positive deer were further south and west and at lower elevation than those where no antibody positive deer were found (Table 2). Associated with these localities were higher mean air and soil temperatures and increased evaporation (Table 2). Indices of temperature and cumulative stress severity also were significantly lower for counties with positive deer.

Based on a discriminant analysis, only one subset of five environmental and climatic variables was useful for distinguishing between counties with and without seropositive deer. The five variables which met the criterion for inclusion in the model and their respective coefficients were elevation, latitude, longitude, mean air temperature, and mean soil temperature in summer. Unstandardized coefficients for the five variables in order of the above listing were 0.005, 0.89, 0.18, 2.85, and −3.10, respectively, with a constant of −16.57. Using the model shown in Table 3 and a prior probability of group measurement of 0.5, we correctly classified 90% of the grouped counties. The group centroids for seropositive and seronegative counties were −0.56 and 2.33, respectively, and the group means were significantly different (Wilks' lambda = 0.43, chi-square = 53.4, $P < 0.0001$) (Fig. 2). If prior probability of group membership was set to 0.19 for seronegative counties and 0.81 for seropositive counties to reflect the actual characteristics of the counties analyzed, the discriminant function using the same five variables accurately grouped 52 of 54 of the seropositive and 11 of 13 of the seronegative counties, with an overall 94% correct classification.

We examined the seven counties misclassified by the prior grouping probability

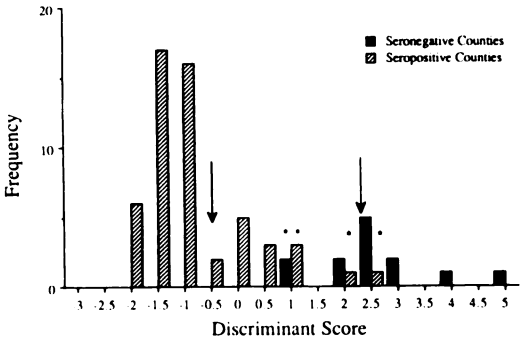

FIGURE 2. Distribution of discriminant scores for counties with and without seropositive deer. Group means are shown by the arrows. Counties or groups of counties misclassified by the final discriminant function with five variables are marked with an asterisk (*).

TABLE 3. Classification table for discriminant analysis distinguishing between counties with and without seropositive deer. Five variables (elevation, latitude, longitude, mean air temperature and mean soil temperature in summer) were included in this model.

Observed county group	Number of counties	Predicted county grouping	
		Seropositive	Seronegative
Seropositive	54	49 (91%)	5 (9%)
Seronegative	13[a]	2 (15%)	11 (85%)

[a] One county excluded from analysis due to missing values.

of 0.5 and failed to find any pattern; the mean number of deer tested per misclassified seropositive county was 13.4 (range 2 to 28) and for misclassified seronegative counties was 15.5 (range 7 to 24). Three of the counties were from Texas, and one each was from Arkansas, Georgia, Illinois, and Tennessee.

DISCUSSION

White-tailed deer in many regions of the eastern United States have been infected with *E. chaffeensis* or a closely related species. The following experimental data support our hypothesis that white-tailed deer are infected with *E. chaffeensis* and may be important in the natural maintenance cycle of this pathogen. These deer are susceptible to infection by *E. chaffeensis* by intravenous inoculation, but not to challenge with *E. canis* (J. E. Dawson, unpubl.). Infected deer circulated rickettsiae for weeks following infection, and *E. chaffeensis* could be cultured repeatedly on DH82 cells (Wellman et al., 1988). Cross-reactions can occur between antibody to different species of *Ehrlichia* in the indirect fluorescent antibody (IFA) test. For example, *E. canis*, the causative agent of canine ehrlichiosis, also occurs in the eastern United States and serologically cross reacts with *E. chaffeensis*. Because of this lack of test specificity, we encourage a cautious evaluation of the potential role of deer as sentinels for human *E. chaffeensis* infection or possibly as reservoirs of this rickettsia. Further studies are required to document natural *E. chaffeensis* infections in wild white-tailed deer by isolation or polymerase chain reaction (PCR) procedures.

The prevalence of deer seropositive to *Ehrlichia spp.* varied in a complex way. Counties sampled from the same state could range from 0% (Madison County, Kentucky) of the deer seropositive to 100% seropositive (Union County, Kentucky). Thus some characteristic associated with exposure may have varied from location to location. One hypothesis to explain observed variation patterns is that a tick vector is responsible for transmission of *Ehrlichia* to deer, and that the distribution of this vector is restricted to some regions of the country or even to specific locations within a state. This hypothesis is biologically plausible as range restrictions have been clearly demonstrated for *Ixodes scapularis* in areas of the eastern United States, such as Maryland and New Jersey (Schulze et al., 1984). In Maryland, infestations of this tick are common in the coastal plain and adjoining Piedmont, but are very rare in the higher elevations of the Appalachian region to the west (Amerasinghe et al., 1992). The distribution of Lyme disease in Maryland and the prevalence of *Borrelia burgdorferi* infection in ticks both reflect the vector distribution and are related to the influences of higher elevation and

colder climate (Amerasinghe et al., 1992). In New Jersey, elevation was the most important environmental variable in explaining *Ixodes scapularis* distribution and density (Schulze et al., 1984). We hypothesize that similar restrictions on vector distribution and abundance may explain our findings with *Ehrlichia* spp. antibody in deer.

Ehrlichia chaffeensis infection may be linked to transmission by *Amblyomma americanum*. Anderson et al. (1993) used PCR to amplify *E. chaffeensis* DNA from *A. americanum*. Thus, *A. americanum* is a good candidate to be a vector of *E. chaffeensis*, although a PCR-positive *Dermacentor variabilis* was collected from an opossum (*Didelphis virginiana*) at Ft. Chaffee, Arkansas (Anderson et al., 1992).

The vertebrate reservoir or reservoirs of *E. chaffeensis* are completely unknown. However, the presence of *A. americanum* in areas with high population densities of white-tailed deer is well established. White-tailed deer can serve as hosts for all three stages of the *A. americanum* and probably have the greatest impact of any host on the abundance of the tick where cattle are not abundant (Patrick, 1976). In a survey of ticks infesting white-tailed deer in 12 southeastern states, *A. americanum* was found on deer in all states surveyed (Smith, 1977).

A major variable influencing the discriminant classification of seropositive counties was the relatively high mean summer soil temperatures which has been shown to contribute to increased survivorship of *A. americanum* and their eggs (Koch and Dunn, 1980). Univariate analyses were repeated, excluding Massachusetts from the data set because of our concern that this northern state with two seronegative counties could unduly influence comparisons. Removing Massachusetts had no effect on the results of analyses indicating the consistency of the associations.

Geometric mean antibody titers were particularly high for deer in Marion County, Mississippi; York County, Virginia; St. Charles County, Missouri; Jones County, Georgia; and Van Buren County, Arkansas. These results may be partly due to increased antigenic stimulus of deer resulting from higher populations of infected ticks. Risk of exposure of humans to infected ticks may also be greater at these sites. The role of deer in the natural history of *E. chaffeensis* requires additional study, but this species may be a sensitive marker of this rickettsia's distribution.

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