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TEMPORAL ASSOCIATION OF *AMBLYOMMA AMERICANUM* WITH THE PRESENCE OF *EHRLICHIA CHAFFEENSIS* REACTIVE ANTIBODIES IN WHITE-TAILED DEER

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ABSTRACT: From 1981 through 1993, tick infestations and serum antibodies reactive to *Ehrlichia chaffeensis*, the causative agent of human ehrlichiosis, were monitored among white-tailed deer (*Odocoileus virginianus*) at Whitehall Experimental Forest, Clarke County, Georgia (USA). Neither ticks nor *E. chaffeensis* antibodies were detected during the first two years of the study. Infestations of the lone star tick (*Amblyomma americanum*), a suspected vector of *E. chaffeensis*, first were noted on deer in 1983. Prevalence and intensity of *A. americanum* sharply increased from 1985 to 1989, and prevalence was 100% from 1990 to 1993. Antibodies reactive to *E. chaffeensis* were first detected in 7% of deer sampled in 1986. Antibody prevalence increased to 21% in 1987 and was 100% from 1988 to 1993. This temporal association between the establishment of *A. americanum* and the appearance of *E. chaffeensis* antibodies provides evidence to support the concept that *A. americanum* could be a natural vector of *E. chaffeensis*. The high prevalence of antibodies among all age classes of deer also reaffirms that white-tailed deer may be sensitive natural sentinels for monitoring the distribution of *E. chaffeensis*.

Key words: *Ehrlichia chaffeensis*, *Amblyomma americanum*, white-tailed deer, *Odocoileus virginianus*, serology, epizootiology.

INTRODUCTION

Human ehrlichiosis is a newly recognized acute febrile illness caused by the rickettsia, *Ehrlichia chaffeensis* (Anderson et al., 1991). The disease ranges from asymptomatic to fatal (Eng et al., 1990) with common clinical symptoms including headache, fever, malaise, and nausea (Fishbein and Dawson, 1990). Since first described in 1986 (Maeda et al., 1987), over 320 human cases of ehrlichiosis have been reported from 27 states, most occurring in the southeastern and midwestern United States (Dawson et al., 1994a).

No vector has been established for *E. chaffeensis*. Many ehrlichiosis patients have a history of tick bites (Fishbein et al., 1989; Harkess et al., 1989). Eng et al. (1990) noted the similarity of geographic distribution between cases of human ehrlichiosis and the range of the lone star tick (*Amblyomma americanum*). Using polymerase chain reaction (PCR) analysis, Anderson et al. (1992, 1993) demonstrated *E. chaffeensis* DNA (gene coding for 16S ribosomal RNA) in adult *A. americanum* from Arkansas, Kentucky, Missouri, New Jersey, and North Carolina (USA), and also from *Dermacentor variabilis* in Arkansas.

Vertebrate reservoir hosts of *E. chaffeensis* are unknown. Serum antibodies reactive to *E. chaffeensis* were detected in 43% of 1,269 white-tailed deer (*Odocoileus virginianus*) in the eastern United States from a geographic area generally corresponding to the distribution of *A. americanum* (Dawson et al., 1994a). White-tailed deer are important hosts for all three life stages of *A. americanum* (Patrick and Hair, 1978; Bloemer et al., 1986, 1988). Based on experimental inoculation of white-tailed deer with *E. chaffeensis* and *E. canis*, deer are susceptible to infection by *E. chaffeensis* but not *E. canis*,

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the causative agent of canine ehrlichiosis (Dawson et al., 1994b). When infected with *E. chaffeensis*, deer developed antibodies and remained rickettsemic through post-inoculation day 24 (Dawson et al., 1994b). Dogs also are susceptible to infection by *E. chaffeensis* (Dawson and Ewing, 1992).

Since 1981, personnel of the Southeastern Cooperative Wildlife Disease Study (SCWDS) have monitored the annual health status of white-tailed deer at Whitehall Experimental Forest (WHEF) (33°-54'N, 83°22'W), Clarke County, Georgia (USA). This work routinely included collection and identification of ticks and retention of frozen serum samples. Serologically, Dawson et al. (1994a) disclosed *E. chaffeensis*-reactive antibodies in deer collected from WHEF between 1989 and 1992, and there are records that lone star ticks became established at WHEF during the period that herd health monitoring was conducted (SCWDS, University of Georgia, Athens, Georgia). Our objective was to determine whether there was a correlation between the presence and intensity of *A. americanum* infestations and the prevalence of *E. chaffeensis*-reactive antibodies in white-tailed deer on the study site.

MATERIALS AND METHODS

Whitehall Experimental Forest is a 325-ha area owned by the D.B. Warnell School of Forest Resources, The University of Georgia, Athens, Georgia, and serves as a research, teaching, and demonstration facility. It is located in the Piedmont physiographic province at the confluence of the North Oconee and Middle Oconee Rivers. The property has a diversity of habitat types including riparian hardwood forests, pine and mixed pine-hardwood upland forests, old fields, and pastures, all of which include a history of human disturbance. White-tailed deer were numerous throughout the study interval with an estimated average density of 37/km² between September 1986 and January 1987 (Litchfield, 1987).

During SCWDS herd health monitoring activities, 110 deer were collected at WHEF from 1981 through 1993. Five deer each were shot in June or July (1981 to 1993) and in November or December (1981 to 1989). Blood was collected by cardiac puncture and allowed to clot. Serum was decanted and stored at −30 C. Ages of deer were determined by tooth replacement and wear (Severinghaus, 1949), and each animal was examined visually for ticks. A subjective estimate of tick infestation intensity was determined for each animal according to the following categories: 0, no ticks observed; 1, fewer than 20 ticks observed; 2, between 20 and 100 ticks observed; and 3, more than 100 ticks observed. Representative specimens of ticks were collected, stored in 70% ethanol, and submitted to the National Veterinary Services Laboratories (U.S. Department of Agriculture, Ames, Iowa, USA) for identification by the keys of Strickland et al. (1976). In addition to the 110 deer examined during SCWDS health monitoring, serum samples and ticks from deer collected by Litchfield (1987) and Van Brackle et al. (1994) were available, including five collected in May 1986, 28 collected in March and April 1987, and 19 collected in March 1992 (serum only).

Antigen for the indirect immunofluorescent antibody (IFA) test was obtained from the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia and fixed in acetone to slides for the IFA procedure (Dawson et al., 1991). Briefly, *Ehrlichia chaffeensis*-infected DH82 canine macrophage cells were suspended in culture medium (Minimum Essential Medium, HyClone Laboratories, Logan, Utah, USA). One drop of the suspension was placed in each well of a 12-well Teflon-coated slide and allowed to air dry for 4 hr. Slides were fixed in acetone for 15 min, air-dried for 30 min, and stored at −70 C. Slides were thawed for 30 min prior to use.

The IFA test was accomplished as described by Dawson et al. (1991). Briefly, beginning at a dilution of 1:64, serum samples were serially diluted in 0.01 M phosphate-buffered saline (PBS) to determine the end-point titer at which fluorescence could be detected using fluorescein isothiocyanate-labelled rabbit anti-deer immunoglobulin G (Kirkegaard and Perry Laboratories Inc., Gaithersburg, Maryland, USA) diluted 1:100 in 0.01 M PBS. Positive results were reported as the reciprocal of the highest dilution at which specific fluorescence of *Ehrlichia* spp. organisms was observed.

Positive control sera were obtained from an experimentally infected white-tailed deer. The deer was immunized with a preparation of live *E. chaffeensis* and bled periodically for 31 days. The deer had a maximum *E. chaffeensis* antibody titer of 1:1,024 27 days post-inoculation (PI). Control serum was obtained from PI day 31 and had a titer of 1:512. Negative control serum was obtained from a hunter-killed fawn from Lake Russell Wildlife Management Area, Stephens County, Georgia. Whole serum from

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TABLE 1. *Amblyomma americanum* infestations and antibodies reactive to *Ehrlichia chaffeensis* in white-tailed deer collected at Whitehall Experimental Forest, Georgia (1981–93).

Year	Number examined	*A. americanum*			*E. chaffeensis* titers		
		Prevalence[a]	Mean intensity[b]	Maximum intensity[b]	Prevalence of titers	Geometric mean titer[c]	Maximum titer
1981	10	0	0.0	0	0	≤63	≤63
1982	10	0	0.0	0	0	≤63	≤63
1983	10	10	0.1	1	0	≤63	≤63
1984	10	10	0.1	1	0	≤63	≤63
1985	10	0	0.0	0	0	≤63	≤63
1986	15	47	0.5	1	7	63+	64
1987	38	87	0.9	1	21	115	512
1988	10	80	0.8	1	100	294	1,024
1989	10	60	0.7	2	100	194	1,024
1990	5	100	1.0	1	100	1,024	2,048
1991	5	100	1.4	2	100	147	512
1992	24	100[d]	2.0[d]	2[d]	100	140	1,024
1993	5	100	1.6	2	100	338	2,048

[a] Prevalence columns are calculated as number of deer with ticks or antibody titers/number of deer sampled, and converted to a percent value.
[b] Tick infestations rated by subjective category scale: 0 = no ticks, 1 = <20 ticks, 2 = 20 to 100 ticks, 3 = ≥100 ticks.
[c] Geometric mean titer values are rounded to nearest whole number; the value 63+ indicate deer had titers of 1:64.
[d] Data available on five of 24 samples.

this animal was negative for antibodies reactive to *E. chaffeensis* by the IFA test.

Prevalence and mean subjective intensity ratings of *A. americanum* were calculated for each deer collection. Prevalence and geometric mean titer (GMT) of serum antibodies reactive to *E. chaffeensis* also were calculated for each deer collection. Seropositive deer were stratified by age class and the GMT was calculated for each class. Analysis of variance was used to test for differences in GMT among age classes and Student's *t*-tests were used to compare GMT's between age classes (Ott, 1993). The chi-square test with Yate's correction factor (Ott, 1993) was used in testing differences in prevalence of *E. chaffeensis*-reactive antibodies in deer collected before *A. americanum* became prevalent at WHEF (1981 to 1986) and after *A. americanum* became prevalent (1987 to 1993). Statistical significance was determined at $P < 0.05$.

RESULTS

Three species of ticks (*A. americanum*, *A. maculatum*, and *Dermacentor nigrolineatus*) were recovered from deer examined at WHEF from 1981 to 1993; however, *A. americanum* was by far the most abundant species and accounted for 390 (99.5%) of 392 ticks identified. Single specimens of *A. maculatum* and *D. nigrolineatus* were identified in collections made in April and December 1987, respectively. Overall, 69 (48%) of 143 deer examined were infested by ticks (Table 1). *Amblyomma americanum* infestations first were detected on deer at WHEF in June 1983 when an adult female was recovered. Infestations remained rare in 1984 and 1985, with only one adult female *A. americanum* being recovered in July 1984. In 1986, the prevalence of *A. americanum* increased dramatically; from 1988 to 1993, all 30 deer examined during June–July collections were infested. The mean intensity of infestation also increased markedly from when *A. americanum* was first found in 1983 until the end of the study in 1993 (Table 1). All life stages of *A. americanum* were found on deer during the June and July collections of 1991 and 1993.

Antibodies reactive to *E. chaffeensis* were not detected among deer at WHEF prior to June 1986 when one of five animals had a titer of 1:64 (Table 1). Four (14%) of 28 deer collected in March and April 1987 had titers of 1:64 to 1:128. The prevalence of seropositive deer abruptly

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TABLE 2. Geometric mean titers (GMT) of *Ehrlichia chaffeensis* reactive antibodies by age group among seropositive white-tailed deer collected from Whitehall Experimental Forest, Georgia, 1981 to 1993.

Age group (years)	Number tested	GMT*	Titer range
<1	8	152.2 A	64–512
1 to 2	33	136.3 A	64–512
3 to 4	14	199.9 A	64–2,048
≥5	11	580.8 B	128–2,048

* Values in column followed by the same letter are not statistically different ($P < 0.05$).

increased to 80% by December 1987; thereafter, seroprevalence was 100% among 59 animals tested (Table 1). The maximum titer detected was 1:2,048 from one deer collected in July 1993 and two deer collected in July 1990. Antibodies reactive to *E. chaffeensis* were detected significantly more often in deer following 1986, when *A. americanum* was widespread at WHEF, than before 1986, when *A. americanum* was rare or absent ($\chi^2 = 56.65$, df = 1, $P < 0.001$).

Based on analysis of variance, there was a significant ($P < 0.001$) difference among GMT values. Using *t*-tests to compare age classes (Table 2), 5-yr-old and older deer had significantly ($P < 0.05$) higher antibody titers to *E. chaffeensis* than younger animals.

DISCUSSION

The distribution of lone star ticks in the southeastern United States is patchy (Smith, 1977), especially those locations with high density tick populations. Although WHEF is within the general geographic range of *A. americanum*, the tick was not observed at WHEF when this study was initiated in 1981. How *A. americanum* reached WHEF is not known. Introduction may have occurred through addition of infested deer to a captive herd maintained at WHEF, through spread from domestic livestock or pets on surrounding land, or by natural spread among wildlife. Natural spread among wildlife is most probable because for many years a high density *A. americanum* population has existed approximately 71 km south of WHEF (Davidson et al., 1994) and gradually has expanded northward over the past decade.

The IFA test applied in this study has been used successfully to survey white-tailed deer for antibodies reactive to *E. chaffeensis* (Dawson et al., 1994a); however, interpretation of data from the IFA test potentially can be confounded by cross-reactions with other *Ehrlichia* spp. (Dawson et al., 1994a). Still white-tailed deer are experimentally susceptible to *E. chaffeensis* infection, become rickettsemic, and produce antibodies detectable with the IFA test (Dawson et al., 1994b). In contrast, attempts to infect white-tailed deer with *E. canis*, which produces cross-reactions on the IFA test, were not successful, and the deer remained negative on the IFA test (Dawson et al., 1994b). Dawson et al. (1994b) also provided strong evidence that antibodies detected among wild deer in our study were not due to *E. canis* cross-reactions.

Although the increasing antibody prevalence with time could be related to antibody decay during storage, we found a high prevalence and equivalent titers in deer serum samples from other locations, including samples from the type location, Fort Chaffee, Arkansas, dating back to 1983 (J.M. Lockhart, unpubl.).

Using transmission studies, ticks have been identified as vectors for other *Ehrlichia* spp., including *Amblyomma americanum* as a vector of *E. ewingii* (Anziani et al., 1990) and *Rhipicephalus sanguineus* as a vector of *E. canis* (Groves et al., 1975). Based on epizootiological evidence, human ehrlichiosis also is a tick-borne disease (Eng et al., 1990; Fishbein et al., 1989; Harkess et al., 1989); *A. americanum* is the principal suspected vector (Anderson et al., 1992, 1993; Dawson et al., 1994a). One of four criteria outlined for confirmation of arthropod vector compentency is field evidence of a significant association of a suspected vector with an appropriate

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vertebrate host (World Health Organization, 1967). We found a temporal association between the establishment of *A. americanum* and the appearance of *E. chaffeensis*-reactive antibodies in white-tailed deer at WHEF; this provides support to the concept that *A. americanum* is a natural vector of *E. chaffeensis*.

Although sample sizes were relatively small, deer in the oldest age class had the highest geometric mean antibody titer to *E. chaffeensis*. An increase in titer with increasing age is evidence of re-exposure and natural boosting of antibody response during annual periods of vector activity. A similar phenomenon has been observed with antibodies to other vector-borne pathogens among white-tailed deer (Fletcher et al., 1991; Mahnke et al., 1993; Stallknecht et al., 1991). That *E. chaffeensis* antibody titers may increase rather than decline with age supports Dawson et al.'s (1994a) belief that white-tailed deer could serve as sensitive natural sentinels for monitoring the distribution of *E. chaffeensis*.

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