Novel *Ehrlichia* Organism (Rickettsiales: Ehrlichieae) in White-Tailed Deer Associated with Lone Star Tick (Acari: Ixodidae) Parasitism

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ABSTRACT Polymerase chain reaction (PCR) evidence of a novel *Ehrlichia* organism was found recently in wild white-tailed deer, *Odocoileus virginianus* Zimmermann, and lone star ticks, *Amblyomma americanum* L., from the southeastern United States. To evaluate whether lone star tick parasitism was associated with the presence of this novel *Ehrlichia* organism in deer, 2 retrospective studies were conducted using specific nested PCR to test archived deer serum samples. The 1st study of 150 serum samples collected from a single deer population over a 15-yr period examined the temporal association between the presence of the *Ehrlichia* organism in deer and parasitism by lone star ticks. The deer *Ehrlichia* was not detected in serum samples collected before 1986, when lone star ticks were absent or rare, but was detected in samples collected in 1986 and every year thereafter, when lone star ticks became increasingly abundant. In the 2nd study, serum samples from 120 deer from 24 sites in 14 southeastern states were tested to evaluate if a site-specific, spatial association existed between the presence of the deer *Ehrlichia* and lone star ticks. All 60 serum samples from the 12 deer populations without evidence of lone star tick infestation were negative for the deer *Ehrlichia*, whereas 83% of the 12 populations infested by lone star ticks had PCR evidence of infection. These data suggest that lone star ticks may be a vector of the deer *Ehrlichia*; however, they do not preclude the involvement of other arthropods in maintaining infection with this organism in deer populations.

KEY WORDS *Ehrlichia, Amblyomma americanum, Odocoileus virginianus,* epidemiology, polymerase chain reaction

HUMAN MONOCYTOTROPIC EHRLICHIOSIS (HME) is an emerging tick-borne disease caused by infection with *Ehrlichia chaffeensis* (Anderson et al. 1991, Dawson et al. 1991). The majority of HME cases have been reported from the southeastern, mid-Atlantic, and south-central regions of the United States (Eng et al. 1990, Fishbein et al. 1994). Current evidence indicates that *E. chaffeensis* is maintained in nature primarily through a cycle involving the lone star tick, *Amblyomma americanum* L., as vector (Anderson et al. 1993, Ewing et al. 1995, Lockhart et al. 1997a) and white-tailed deer, *Odocoileus virginianus* Zimmermann, as reservoir host (Dawson et al. 1994, Lockhart et al. 1997a). This cycle has been demonstrated experimentally by transstadial transmission of *E. chaffeensis* by lone star ticks (Ewing et al. 1995). A 2nd ehrlichial disease, human granulocytotropic ehrlichiosis (HGE), is more common in the northeastern and midwestern United States. The unnamed HGE agent is thought to be transmitted by the black-legged tick, *Ixodes scapularis* Say (Pancholi et al. 1995, Telford et al. 1996), the adults of which are commonly found on white-tailed deer (Strickland et al. 1981).

Recently, Dawson et al. (1996) reported evidence of a 16S rRNA gene fragment characteristic of a novel ehrlichial organism in white-tailed deer from Georgia and Oklahoma. Sequence analysis revealed this novel deer *Ehrlichia* was most closely allied with the *Ehrlichia phagocytophila* genogroup, which also includes *Ehrlichia equi, Ehrlichia platys,* and the HGE agent. In fact, polymerase chain reaction (PCR) primers designed to amplify the HGE agent will also amplify the deer *Ehrlichia* (Little et al. 1997). Subsequent work showed the deer *Ehrlichia* is widespread among deer in the southeastern United States (Little et al. 1997) and PCR assays also detected this same organism in adult lone star ticks (Lockhart et al. 1997b). Although not known to cause human disease, the presence of the deer *Ehrlichia* in white-tailed deer and lone star ticks may confound epidemiological studies of the agents of human ehrlichioses.

Two previous retrospective studies disclosed a temporal and spatial association between lone star tick parasitism and the presence of *E. chaffeensis*-reactive antibodies in white-tailed deer (Lockhart et al. 1995,

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1996). We used PCR to test archived samples to determine if similar temporal and spatial associations exist between the lone star tick and the novel deer *Ehrlichia*.

Materials and Methods

Sample Collection. Most serum samples and ticks used in these studies were collected for the temporal and spatial association studies by Lockhart et al. (1995, 1996). These samples and some additional ones were collected from white-tailed deer as previously described (Lockhart et al. 1995, 1996). Serum samples were stored at −20°C after collection. Representative ticks were placed in 70% ethanol and submitted to the National Veterinary Services Laboratories (USDA, Ames, IA) for identification according to the keys of Strickland et al. (1976).

Serum samples from at least 5 deer from each time point or site were tested by PCR. In the temporal association study, 150 sera collected from a single deer population at Whitehall Experimental Forest (WHEF) in Clarke County, GA, from 1982 to 1997 were tested by PCR. Of the samples tested, 105 were a subset of the 162 tested by Lockhart et al. (1995) for *E. chaffeensis;* an additional 45 sera, collected from 1993 through 1997, also were tested. As a positive control for potential false negative results caused by long-term storage, 10 sera collected from Ossabaw Island, Chatham County, GA, in 1982 ($n = 5$) and 1995 ($n = 5$), where lone star ticks have consistently been observed on deer, also were tested.

In the spatial association study, 120 of the 300 sera analyzed by Lockhart et al. (1996) for *E. chaffeensis* were chosen from 24 deer populations in 14 southeastern states (Fig. 1). Sites were spread across the southeastern United States and included 12 populations where lone star tick infestations had been previously identified and 12 where lone star tick infestations were believed absent or rare. Populations at the following locations were selected: Alabama—G. E. Property, Lowndes County; Lee Haven Property, Sumter County; Arkansas—Big Lake National Wildlife Refuge, Mississippi County; Fort Chaffee, Sebastian County; Florida—Everglades National Park, Broward County; St. Marks National Wildlife Refuge, Wakulla County; Georgia—Chickamauga National Battlefield, Walker County and Catoosa County; Fort Benning, Muscogee and Chattahoochee County; Kentucky—Mammoth Cave, Edmonson County; Louisiana—Lake Ophelia National Wildlife Refuge, Avoyelles Parish; Weldon Property, Claiborne Parish; Maryland—Catoctin Mountain National Park, Frederick County; Blackwater National Wildlife Refuge, Dorchester County; Mississippi—Panther Swamp National Wildlife Refuge, Yazoo County; Missouri—Squaw Creek National Wildlife Refuge, Holt County; Knob Noster State Park, Johnson County; North Carolina—Pee Dee National Wildlife Refuge, Anson County; Alligator River National Wildlife Refuge, Dare County; South Carolina—Croft State Park, Spartanburg County; Cape Romain National Wildlife Refuge, Charleston County; Tennessee—Hatchie National Wildlife Refuge, Haywood County; Cross Creeks National Wildlife Refuge, Stewart County; Virginia—Prince William Forest National Park, Prince William County; and West Virginia—Bluestone Farm, Monroe County.

PCR Test of Serum. Extraction of DNA from 20 μl of deer serum was performed using InstaGene Purification Matrix (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. For each batch of samples tested, an Ossabaw Island serum sample that consistently tested positive was included as a positive control, and molecular biology-grade water (J. T. Baker, Phillipsburg, NJ) as a negative control. DNA extracted from deer serum was used as template in a diagnostic nested PCR, which amplifies a characteristic fragment of the 16S rRNA gene of the deer *Ehrlichia* as previously described, but not the HGE agent (Little et al. 1997). Amplicons were separated by electrophoresis in a 1% agarose gel and visualized by ethidium bromide staining and UV transillumination. To avoid contamination, DNA extraction, primary reactions, secondary reactions, and evaluation of samples on agarose gels each were performed in different areas.

Analysis of Data. Tick prevalence was calculated for each time point and area tested. The chi-square test with the Yates correction factor (Ott 1993) was used to evaluate significance between the presence of the deer *Ehrlichia* and the presence of lone star ticks in the temporal association study and the site-specific study. Statistical significance was considered at $P < 0.01$.

Results

No evidence of infection with the deer *Ehrlichia* was found in serum samples from WHEF before 1986, a time when lone star ticks were absent or rarely observed at this location. However, this organism was detected in serum samples from WHEF after lone star ticks were observed and began to increase in prevalence. With the exception of 1988, all years subsequent to 1986 had at least 1 deer that tested PCR positive for the deer *Ehrlichia* (Table 1). This organism also was detected by PCR in deer serum samples from Ossabaw Island archived since 1982 (4/5, 80%) and in samples from 1995 (5/5, 100%). The chi-square test with the Yates correction factor demonstrated a significant ($\chi^2 = 43$, $P < 0.01$) association between the presence of lone star ticks and the presence of the deer *Ehrlichia*.

All serum samples from the 12 deer populations not parasitized by lone star ticks were PCR-negative for the deer *Ehrlichia*. In contrast, PCR detected the deer *Ehrlichia* in 10 of 12 (83%) deer populations with lone star tick infestations. The sites with lone star ticks but where PCR evidence of the deer *Ehrlichia* was not found were Fort Chaffee, Sebastian County, AR, and Cape Romain National Wildlife Refuge, Charleston County, SC, (Table 2). The Yates corrected chi-square test showed a significant ($\chi^2 = 59$, $P < 0.01$) association between the presence of lone star ticks in a geographic site and PCR evidence of infection with the deer *Ehrlichia*.

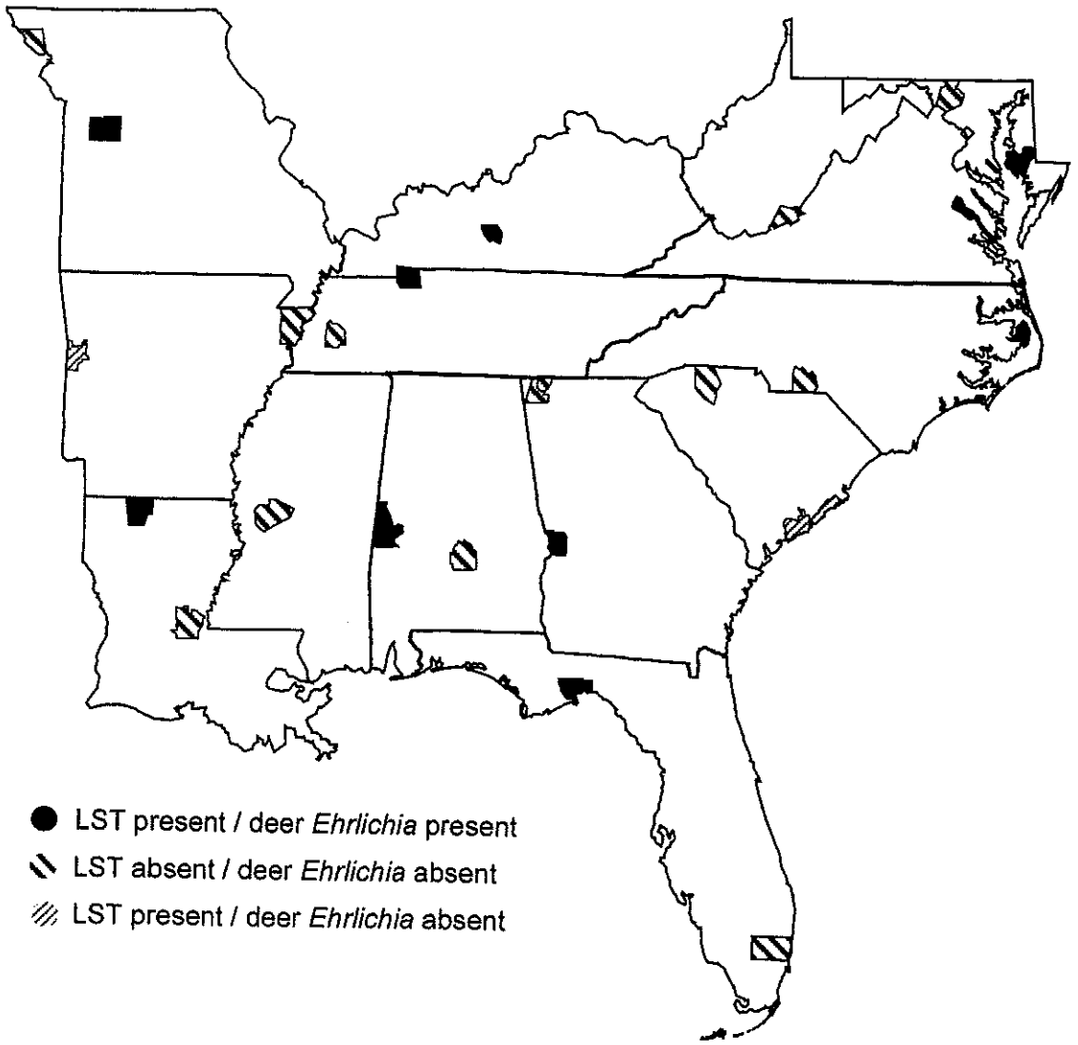

Fig. 1. Map of the southeastern United States showing the geographic distribution of sites chosen for the site-specific study. Sites shown include those with evidence of both lone star tick (LST) and deer *Ehrlichia*, without lone star tick or deer *Ehrlichia*, and with lone star tick but without deer *Ehrlichia*. No sites were identified without lone star tick that had deer *Ehrlichia*.

Discussion

The 2 companion retrospective studies reported here show that PCR evidence of infection with the deer *Ehrlichia* in white-tailed deer is temporally and spatially associated with lone star tick parasitism. These data fulfill 1 of the 4 criteria outlined for confirmation of arthropod vector status (WHO 1967) and indicate that the lone star tick may be a vector of the deer *Ehrlichia*. White-tailed deer are a natural reservoir host for *E. chaffeensis* (Lockhart et al. 1997a), and the lone star tick is considered to be the principal vector (Anderson et al. 1992, 1993; Ewing et al. 1995). Our findings closely parallel those of Lockhart et al. (1995, 1996), where serologic evidence that deer were infected with *E. chaffeensis* was associated with the presence of lone star ticks on those deer. Our data suggest that, like *E. chaffeensis*, infection with the deer *Ehrlichia* also may be maintained in deer populations by lone star tick parasitism. However, the possibility that another arthropod with a similar range may be involved in the transmission of this organism cannot be excluded.

Additionally, our studies contribute to delineating the geographic distribution of the deer *Ehrlichia* within the southeastern United States. Prior work showed the deer *Ehrlichia* was present in Arkansas, Georgia, Oklahoma, and South Carolina (Dawson et al. 1996, Little et al. 1997). The data reported here expand the known range of this organism to include 9 additional states—Alabama, Florida, Kentucky, Loui-

Table 1. Lone star tick infestations and PCR evidence of the deer *Ehrlichia* in white-tailed deer collected from Whitehall Experimental Forest, Georgia, 1982–1997

Year	Ticks[a]		Deer *Ehrlichia*	
	No. deer examined[b]	% prevalence[c]	No. deer tested[d]	% prevalence[c]
1982	10	0	5	0
1983	10	10	5	0
1984	10	10	5	0
1985	10	0	5	0
1986	15	47	15	7
1987	38	87	20	5
1988	10	80	10	0
1989	10	60	10	50
1990	5	100	5	20
1991	5	100	5	80
1992	5	100	15	27
1993	5	100	15	33
1994	5	100	15	80
1995	10	90	6	100
1996	9	100	9	89
1997	5	100	5	60

[a] Data for tick prevalence from 1982 to 1993 from Lockhart et al. (1995); all (99.5%) but 2 ticks were *A. americanum* (Lockhart et al. 1995). Data from 1993 to 1997 were derived from additional deer not included in Lockhart et al. (1995); all of these ticks were *A. americanum*.

[b] From 1982 to 1989, 5 deer each were collected in June or July and in November or December; additional deer were collected in May 1986 and March and April 1987; from 1990 to 1993 and in 1996 all deer were collected in June or July; in 1994, 1995, and 1997, deer were collected in January, September, and March, respectively.

[c] Prevalence was calculated as number of deer with ticks or PCR-positive for the deer *Ehrlichia* divided by the total number of deer sampled and converted to a percentage value.

[d] In some years, the number of deer tested for deer *Ehrlichia* was fewer or greater than the number of deer examined for ticks.

Table 2. Lone star tick infestations and results of PCR testing for the deer *Ehrlichia* in white-tailed deer collected from the southeastern United States, 1982–1997

Area[a]	Collection date	Positive test[b]	% prevalence[c]
Lone star tick positive sites			
Lee Haven Property, AL	July 1988	4/5	80
Fort Chaffee, AR	Aug. 1983	0/5	0
St. Mark's NWR, FL	Sept. 1987	1/5	20
Fort Benning, GA	Aug. 1990	1/5	20
Mammoth Cave NP, KY	Sept. 1984	3/5	60
Weldon property, LA	Sept. 1987	4/5	80
Blackwater NWR, MD	July 1993	4/5	80
Knob Noster SP, MO	Sept. 1992	3/5	60
Alligator River NWR, NC	Sept. 1985	1/5	20
Cape Romain NWR, SC	July 1987	0/5	0
Cross Creeks NWR, TN	Aug. 1995	4/5	80
Prince William Forest NP, VA	Aug. 1988	3/5	60
Lone star tick negative sites			
G. E. property, AL	Aug. 1990	0/5	0
Big Lake NWR, AR	Sept. 1992	0/5	0
Everglades NP, FL	July 1982	0/5	0
Chickamauga NB, GA	Aug. 1991	0/5	0
Lake Ophelia NWR, LA	Sept. 1992	0/5	0
Catoctin Mountain NP, MD	Aug. 1988	0/5	0
Panther Swamp NWR, MS	Sept. 1986	0/5	0
Squaw Creek NWR, MO	Sept. 1992	0/5	0
Pee Dee NWR, NC	July 1996	0/5	0
Croft SP, SC	Aug. 1989	0/5	0
Hatchie NWR, TN	Sept. 1994	0/5	0
Bluestone Farm, WV	Aug. 1987	0/5	0

[a] NB, National Battlefield; NP, National Park; NWR, National Wildlife Refuge; SP, State Park.

[b] Number of deer PCR positive for the deer *Ehrlichia* over the number of deer tested.

[c] Prevalence was calculated as number of deer with ticks or PCR-positive for the deer *Ehrlichia* divided by the number of deer sampled and converted to a percentage value.

siana, Maryland, Missouri, North Carolina, Tennessee, and Virginia. Although the deer *Ehrlichia* is not known to cause human disease, our data suggest that humans in these endemic areas may be exposed to this organism.

Direct evidence of the deer *Ehrlichia* in *A. americanum* has been found by testing pools of wild-caught adult lone star ticks (Lockhart et al. 1997b), but the prevalence of infection was low (minimum infection frequency = 1.71%). The low prevalence in lone star ticks and the fact that the deer in our site-specific study were examined predominately in the summer months leaves open the possibility that other ticks, such as *Ixodes scapularis* and *Amblyomma maculatum*, whose ranges overlap that of *A. americanum* might be important vectors of the deer *Ehrlichia*. This is improbable because in our temporal study, a high prevalence of the deer *Ehrlichia* was detected at a site where *I. scapularis* and *A. maculatum* are rare or absent and only after the arrival of lone star ticks in the mid-1980s.

Because *Ehrlichia* spp. are intracellular organisms, whole blood or white blood cell preparations are preferred over serum for extracting template DNA for PCR tests. However, the fact that DNA of some *Ehrlichia* spp. (Little et al. 1999, CDC 1995) and of other organisms (Sandford and Paré 1997) can be amplified from serum samples is advantageous to epidemiological studies because serum is often archived and thus readily available for retrospective studies. Although repetitive freezing and thawing is sometimes thought to decrease the utility of samples for diagnostic testing, our results indicate that these samples still can be of great value. Our finding of DNA characteristic of the deer *Ehrlichia* in serum samples collected from Ossabaw Island in 1982 supports the validity of using PCR to test archived serum samples for this organism.

To date, evidence of 3 species of *Ehrlichia* (*Ehrlichia chaffeensis*, the HGE agent, and the deer *Ehrlichia*) has been reported from white-tailed deer (Dawson et al. 1996, Lockhart et al. 1997a, Little et al. 1998), although only *E. chaffeensis* has been confirmed in deer by isolation (Lockhart et al. 1997a). Culture isolation of *Ehrlichia* spp. is difficult and often unsuccessful using field samples (Dawson et al. 1996). However, isolation of the deer *Ehrlichia* will be essential to characterize this organism fully and to understand any interactions it may have with other *Ehrlichia* spp. For example, fundamental questions remain concerning the effect of coinfection of deer with multiple *Ehrlichia* spp. on such factors as protective immunity, enhanced pathology, or competitive exclusion between related organisms. Our data suggest that the deer *Ehrlichia* may occur in the same tick vector–vertebrate reservoir cycle as *E. chaffeensis*. Future epidemiolog-

ical studies should consider the potential influence of interactions between related species on the natural history of the agents of human ehrlichioses.

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