JOURNAL OF THE TENNESSEE ACADEMY OF SCIENCE 82(3–4):57–61 JULY–OCTOBER 2007

BORRELIA INFECTION RATES IN WINTER TICKS (*DERMACENTOR ALBIPICTUS*) REMOVED FROM WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) IN CHEATHAM COUNTY, TENNESSEE

BRIDGET M. SHARIAT, JENNIFER A. FREIMUND, STEPHEN M. WRIGHT, C. STEVEN MURPHREE, AND JENNIFER T. THOMAS

Department of Biology, Belmont University, Nashville, TN 37212 (BS, CSM, JTT)
Department of Biology, Middle Tennessee State University, Murfreesboro, TN 37132 (JAF, SMW)

ABSTRACT—White-tailed deer, *Odocoileus virginianus*, are significant reservoir hosts of *Borrelia burgdorferi*, the causative agent of Lyme disease. Hard ticks serve as vectors of both *B. burgdorferi* and *Borrelia lonestari*, a species reported from the southeastern United States and associated with Southern Tick Associated Rash Illness or STARI. Winter ticks, *Dermacentor albipictus*, were collected from hunter-killed deer in Cheatham County, Tennessee in October and November of 2005. Six of 18 (33%) pooled tick samples tested positive for *Borrelia* species; two of six were identified as *B. burgdorferi* and four as *B. lonestari*. This investigation demonstrates that both *B. burgdorferi* and *B. lonestari* are present in *O. virginianus* populations in Cheatham County, Tennessee. It is noteworthy that *D. albipictus* is capable of harboring *Borreliae* from infected white-tailed deer. This is the first report of *Borrelia* detection in *D. albipictus* in Tennessee.

Lyme disease is the most frequent vector-borne illness in the United States (Goddard, 2001). Bites from infected ticks are followed by flu-like symptoms such as fever, chills, headache, muscle aches, vomiting and fatigue. Later complications can include meningitis, paralysis, irregular heartbeat, and painful, enlarged joints. Many individuals with Lyme disease develop a large red rash resembling a bull's eye at the bite location called erythema migrans. (CDC, 1997).

One-half of all United States Lyme Disease cases are transmitted to humans via the black-legged deer tick, *Ixodes scapularis* Say (CDC, 1997). The black-legged deer tick is found in much of the eastern United States and is a multi-host tick with larvae and nymphs preferring mice. Adult ticks prefer white-tailed deer, *Odocoileus virginianus* Zimmerman, which serves as the primary reproductive host (Wildlife Control, 2006). Additionally, regions of lush eastern forest contain not only high populations of deer, but also of *I. scapularis* (Magnarelli et al., 1995). All life cycle stages of the black-legged deer tick can harbor the causative agent of Lyme disease. Humans most commonly become infected when bitten by nymph or adult *I. scapularis* (CDC, 2005).

The etiology of Lyme disease in the southern United States is less well understood even though cases are reported annually. Many patients present with symptoms of Lyme disease in southern states but test negative for the presence of the spirochete. Only a small percentage of southern ticks have been reported to be infected with spirochetes of *Borrelia* spp.; however, there have been isolations of *B. burgdorferi* Johnson from both rodents and ticks in various southern states (Barbour et al., 1996). Another bacterium, *Borrelia lonestari* Barbour, has been identified from the lone star tick (*Amblyomma americanum* (L.)) in the southeastern United States (Barbour et al., 1996). This bacterium is reported to be responsible for a condition known as Southern Tick Associated Rash Illness or STARI, since the causative agent of Lyme disease, *B. burgdorferi*, remains rare in southern states (CDC, 2005). Another common tick associated with white-tailed deer in middle Tennessee is the winter tick (*Dermacentor albipictus* Packard). Although all life stages of this species occur on deer, it is reasonable to test these one-host ectoparasites for the presence of *Borrelia* species that could be transmitted to deer by multi-host tick vectors. While a related species, *D. variabilis*, has been shown in a laboratory setting to support *B. burgdorferi* replication, (Soares et al., 2006), *Borrelia* detection in *D. albipictus* has not been reported from Tennessee.

The purpose of this study was to determine whether winter ticks could harbor the *Borrelia* species that cause Lyme disease and STARI by testing nymphal and adult specimens collected from hunter-killed deer from Cheatham County, Tennessee.

MATERIALS AND METHODS

Ticks were collected from hunter-killed deer at the Tennessee Wildlife Resources Agency check station in Cheatham County, Tennessee on October 29 and November 11, 2005. Eighty eight ticks were collected from 19 deer, placed in vials containing 70% ethanol, and stored at 4°C until processing. All ticks were identified and determined to be *D. albipictus* (Brinton et al., 1965). Gender, weight, age, and area within the Cheatham Wildlife Management Area for each deer are provided in Table 1. Using these data, the ticks were separated based on deer location and life cycle stages. Nymphs and both engorged and unengorged adults were later removed and pooled into 18 vials for DNA isolation (Table 2). Pools were prepared as: 8 nymphs, 4 unengorged adults, or 3 engorged adults.

TABLE 1. Data collected from hunter-killed deer checked at the Cheatham Wildlife Management Area (WMA) checking station in October and November, 2005. Areas 1, 4, 5, 7, 10, 12, 13, 14, 15, 16 and 18 within the Cheatham WMA may be viewed at: http://www.state.tn.us/twra/gis/wmapdf/Cheathambw.pdf.

	Deer	Gender	Weight (lbs)	Age (yrs)	Area
10/29/05 Collection	1	Male	137.2	1.5	13
	2	Male	83.8	1.5	7
	3	Male	97.2	2.5	5
	4	Male	102	1.5	Out of area
	5	Female	82	1.5	18
	6	Male	77	1.5	18
	7	Female	74	1.5	18
	8	Male	156	2.5	Out of area
	9	Female	87	2.5	10
	10	Male	81	1.5	15
11/5/05 Collection	11	Female	83	2.5	4
	12	Male	119	3.5	1
	13	Female	85	4.5	1
	14	Male	105	1.5	18
	15	Female	89	3.5	12
	16	Male	108	2.5	12
	17	Male	131	3.5	16
	18	Male	112	2.5	14
	19	Female	88	4.5	14

A Genomic DNA II kit (Zymo Research, Orange, California) and a modified protocol established by Haynes et al. (2005) were used to extract DNA from ticks. Briefly, ticks were cut using a sharp blade and placed in an extraction tube. To each extraction tube sample, 500 µL of Genomic Lysis Buffer was added and a battery-powered homogenizer was used for two to three minutes to ensure the tick gut contents were released. The tubes were allowed to stand 10–15 min at room temperature, and the volume of each tube was loaded into a new collection tube assembled with a Zymo Spin Column. This assembly was microcentrifuged at 12,000 rpm for one min. To each column 500 µL of DNA Wash Buffer was added and then spun at 12,000 rpm for one min. This wash was repeated and the column was placed into a new 1.5 mL tube. Thirty µL of heated Elution Buffer (45°C) was added to each column and allowed to stand for one min. After each tube was spun for 10 sec, the eluted DNA was stored in a −20°C freezer prior to amplification.

The 18 DNA samples were tested for the presence of *Borrelia* species by the polymerase chain reaction (PCR). The tick extract was tested using primers that amplify the flagellin gene for both *B. burgdorferi* and *B. lonestari* (Re et al., 2004). An initial amplification was followed by nested PCR, which provides more definitive distinction between positive and

TABLE 2. Tick Pools used for DNA Evaluation.

Single[a] Host	Description	# Ticks	Multiple Hosts	Description	# Ticks
N1 [1]	nymphs from deer 12	8	N1 [3]	nymphs from deer 2,6,7,9	8
N2 [2]	nymphs from deer 18	8	N2 [4]	nymphs from deer 8,10	8
			N3 [5]	nymphs from deer 14,15,16,17,19	8
A1 [6]	adults from deer 4	4	A1 [11]	adults from deer 2,6,9,10	4
A2 [7]	adults from deer 12	4	A2 [12]	adults from deer 11,13,15,18	4
A3 [8]	adults from deer 17	4			
A4 [9]	adults from deer 14	4	AE1 [16]	adults from deer 4,5	3
A5 [10]	adults from deer 16	4	AE2 [17]	adults from deer 1,2,3	3
AE1 [13]	adults from deer 12	3	AE3 [18]	adults from deer 15,16,18,19	5
AE2 [14]	adults from deer 17	3			
AE3 [15]	adults from deer 14	3			

[a] N = nymph; A = adults unengorged; AE = adults engorged, [ ] = sample number.

negative samples than a single amplification. All PCR reagents (*Taq* polymerase, 10 X buffer, dNTPs) were obtained from Promega (Madison, Wisconsin). Primers used for primary amplification were: Forward, 5′ CAAAAATTAATACACCAGCAT; Reverse, 5′ GCAATCATAGCCATTGCAGA. Primers used for nested amplification were: Forward: 5′ CTAATGTTGCAAATCTTTT; Reverse, 5′ GCATCTTTAATTTGAGCATA. All primers were prepared by Integrated DNA Technologies (Coralville, Iowa). A negative control, which includes all reagents, substituting sterile deionized H_2O for tick DNA, was included and verified contamination did not occur. The PCR products were frozen at −20°C prior to gel electrophoresis.

Nested PCR products were loaded into the wells and electrophoresed in a 1.25% agarose gel (NuSieve, FMC Bioproducts, Rockland, Maine) with TAE (Tris-Acetate-EDTA) buffer. Each gel included a standard ladder, BioMarker Low (BioVentures, Murfreesboro, Tennessee). The gel was stained with ethidium bromide and visualized with UV light. Images were taken with a Kodak EDAS 290 gel imaging system (Rochester, New York).

Samples that demonstrated a band on the gels were prepared for hybridization with probes specific for sequences characteristic of *B. burgdorferi* or *B. lonestari*. Nested PCR products were ethanol precipitated and applied to an epoxy functionalized substrate using a SpotBot microarrayer according to manufacturer instructions (TeleChem International, Sunnyvale, California). Following DNA spotting, the slide was heated (95°C, 25 min), exposed twice to ultraviolet light (6500 J/cm^2) and washed (0.1% SDS, 3 min and deionized H_2O, 2 min). After blocking for 1 hour (ArrayIt Blocker, TeleChem) and performing identical SDS and dH_2O washes, the slide was exposed to 95°C dH_2O for 2 min to denature the DNA. A probe mixture was prepared according to manufacturer specifications at a final concentration of 250 ng/each probe in hybridization buffer (UniHyb, TeleChem). The probe sequence specific for *B. burgdorferi* was 5′ ATCTATAAAGAATAGTACTGAG and for *B. lonestari* was 5′ TCCAGCTCAAGGTGGGATTAGC. The *B. burgdorferi* probe was labeled with Cy5 and the *B. lonestari* probe was labeled with FITC. Probes were prepared by Integrated DNA Technologies. Hybridization was done at 56°C for 45 min. The slide was washed in increasingly stringent washes (2 X SSC, 0.1% SDS; 0.2 X SSC, 0.1% SDS; 0.2 X SSC) for 2 min each. The slide was scanned with a Genetix laser scanner (Queensway, United Kingdom) following the third SSC wash.

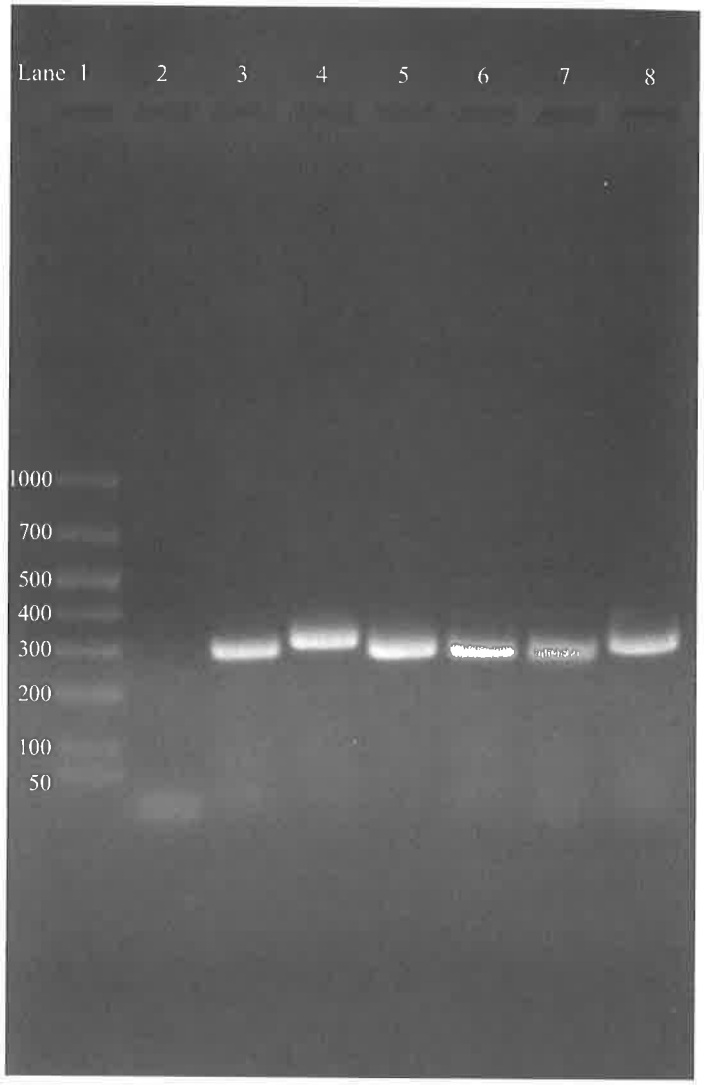

FIG. 1. Gel electrophoresis of amplified tick extracts. The standard ladder (BioMarker Low) appears on the far left in lane 1. Fragment sizes are 1000, 700, 525/500, 400, 300, 200, 100, and 50 bp. Lane 2 contains the negative control; lane 3, sample 2; lane 4, sample 3; lane 5, sample 4; lane 6, sample 11; lane 7, sample 12; lane 8, sample 16. The predicted fragment size for *Borelia burgdorferi* is 319 bp and 298 for *B. lonestari*.

RESULTS

Detection of *Borrelia* species occurred in six of 18 pools. A photograph of the gel is shown in Fig. 1. The bands indicating *Borrelia* are located at 319 base pairs (bp), signifying *B. burgdorferi*, or 298 bp, representing *B. lonestari*. Samples 2, 4, 11 and 12 migrated as expected for *B. lonestari* (Fig. 1). Samples 3 and 16 are slightly larger on the gel, suggesting a PCR product characteristic of *B. burgdorferi*. The negative control sample did not contain DNA and did not produce a band, verifying that contamination did not occur.

Verification of *Borrelia* identity is evident in Fig. 2. The probe specific for *B. burgdorferi* bound to the positive *B. burgdorferi* control, and samples 3 and 16 (the Cy5 label appears red). The *B. lonestari* probe, blue, bound to the positive control and samples 2, 4, 11 and 12. No binding was evident with the negative control. Of the 18 samples tested, two were determined to be *B. burgdorferi* and four were characterized as *B. lonestari* (Table 3). When comparing host deer from which positive tick samples were obtained (Tables 1 and 2), it is likely that two deer, 5 and 7, were infected by *B. burgdorferi* and two other deer, 10 and 18, were infected by *B. lonestari*.

DISCUSSION

Previous studies in southeastern states that attempted to detect *Borrelia* species in *Dermacentor albipictus* were unsuccessful (Luckhart et al., 1991,1992; Taft et al., 2005). This study represents the first report of the presence of any *Borrelia* species detected by PCR analysis in *D. albipictus*. Researchers that reported *Borrelia* in the American dog tick, *Dermacentor*

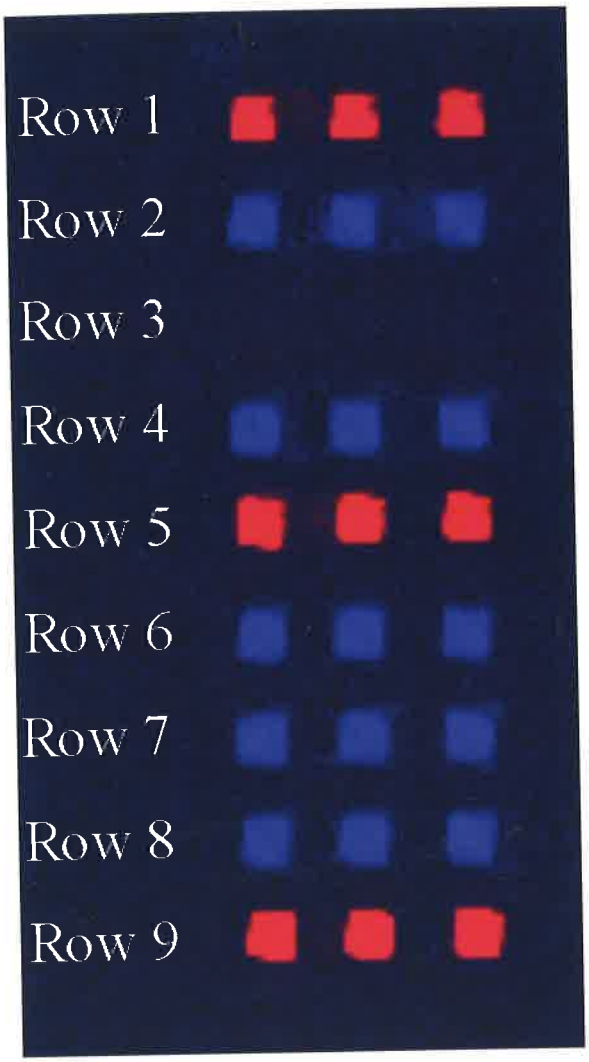

FIG. 2. Differentiation of *Borrelia* species based on specific probe hybridization. Each sample was applied to the substrate in triplicate as a microarray. The probe specific for *B. burgdorferi* sequences was labeled with Cy5 and which appears red. The probe specific for *B. lonestari* was labeled with FITC and appears blue. From the top, the first row is a positive control for *B. burgdorferi*. The second row is a positive control for *B. lonestari*. The third row, appearing blank, was spotted with the negative control. PCR products from each sample were spotted in the remaining rows: row 4 contains DNA from sample 2 (blue, indicating sequences characteristic of *B. lonestari*); row 5, sample 3 (red, *B. burgdorferi* sequences); row 6, sample 4 (blue); row 7, sample 11 (blue); row 8, sample 12 (blue); row 9, sample 16 (red).

variabilis, (Stromdahl et al., 2001), a close relative of the winter tick, failed to detect it in *D. albipictus.*

Because *Borrelia* specific primers were used for the flagellin gene where *B. lonestari* is 21 bp smaller than *B. burgdorferi*, it was possible to suggest differentiation between the *Borreliae* based on electrophoretic migration. Absolute identification was accomplished using probes that recognized and bound the sequence specific for each *Borrelia* species (Fig. 2). Out of the 18 samples tested, six (33.3 %) tested positive for a *Borrelia* species. Two of the six were identified as *B. burgdorferi* and four as *B. lonestari*. Additional insights are

TABLE 3. Tick pool samples and likely deer host positive for *Borrelia* species.

Borrelia species	Pool Number	% Positive	Deer Host
B. burgdorferi	3, 16	11.1	5, 7
B. lonestari	2, 4, 11, 12	22.2	10, 18

evident when considering the origin of each sample (Table 2). Sample 2, positive for *B. lonestari*, represents ticks taken from deer 18. Pooled sample 12, also positive for *B. lonestari*, included deer 18, suggesting this deer was responsible for infection of both tick pools. Similarly, pooled samples 4 and 11 each included deer 10 and both pools were documented to contain *B. lonestari*. One *B. burgdorferi* positive pool (sample 3) included ticks taken from deer 2, 6, 7, and 9. Other tick samples collected from deer 2, 6, and 9 remained negative for *B. burgdorferi*, suggesting a single deer, number 7, was responsible for the infected tick pool. The second pool positive for *B. burgdorferi* (sample 16) represented ticks taken from deer 4 and 5. Since sample 16 contained ticks from deer 4 and remained negative, it would appear that deer 5 was infected. The locations of specific samples failed to demonstrate a distinct pattern concerning which deer were infected with *Borrelia*, although both deer harvested from area 18 were positive for *B. burgdorferi*. There is not enough consistency among tick samples to make any correlations among gender, weight or age of the infected deer. However, it is likely that *O. virginianus* plays an important role in the epidemiology of Lyme disease and STARI. It has been suggested that deer may serve as a reservoir host for *B. lonestari* (Moyer et al., 2006).

The success of this investigation is due in part to the collection method used. Recovering ticks from large mammals, such as deer, is infrequent, and recovering fully engorged ticks of any species from deer is still more infrequent. Instead of taking samples of deer blood, determination of whether deer were infected was evaluated by testing *D. albipictus* for *Borrelia* species. Although the infection rates of deer reported in this study are relatively high, it is unlikely that *D. albipictus* would transmit *Borrelia* species to humans or other animals. Winter ticks are one-host ticks that remain on deer for their entire life cycle. An important finding of this study is that blood-engorged *D. albipictus* removed from deer can be used to demonstrate *Borrelia* infection. Additional research is needed to determine the length of time that *D. albipictus* can maintain *Borrelia* species after feeding on infected deer. This investigation demonstrates both *B. burgdorferi* and *B. lonestari* are present in *O. virginianus* populations in Cheatham County, Tennessee. It is particularly noteworthy that, for the first time, *D. albipictus* has been documented as harboring *Borrelia* species from infected white-tailed deer in middle Tennessee.

ACKNOWLEDGEMENTS

Thanks to R. Cromer, Cheatham Wildlife Management Area, Tennessee Wildlife Resources Agency for assisting with sample collection at the check station. Thanks to J. Jones, GIS Manager at the Tennessee Wildlife Resources Agency Nashville Office for adding area designations to the

online map of the Cheatham WMA. This work was supported, in part, by grant #0216716 from the National Science Foundation.

Literature Cited

BARBOUR, A. G., G. O. MAUPIN, G. J. TELTOW, R. J. CARTER, AND J. PIESMAN. 1996. Identification of an uncultivable *Borrelia* species in the hard tick *Amblyomma americanum*: possible agent of a Lyme-like illness. J. Infect. Dis., 173:403–409.

BRINTON, E. P., D. E. BECK, AND D. M. ALLRED. 1965. Identification of the adults, nymphs and larvae of ticks of the genus *Dermacentor* Koch (*Ixodidae*) in the western United States. Brigham Young Univ. Sci. Bull. Biol. Series., Vol. V. (4). 44 pp.

CENTERS FOR DISEASE CONTROL AND PREVENTION. 1997. Case definitions for infectious conditions under public health surveillance. MMWR, 46:1–55.

———. 2005. CDC Lyme Disease Home Page. Available via the Internet (www.cdc.gov/ncidod/dvbid/lyme/index.htm).

GODDARD, J. 2001. What's going on with Lyme disease in the South? Infect. Med., 18:132–133.

HAYNES, J. M., P. P. LEE, R. S. SEIPELT, AND S. M. WRIGHT. 2005. Detection of *Borrelia burgdorferi* sequences in a biopsy from a Tennessee patient. J. Tennessee Acad. Sci., 80:57–59.

LUCKHART, S., G. R. MULLEN, AND J. C. WRIGHT. 1991. Etiologic agent of Lyme disease, *Borrelia burgdorferi* detected in ticks (Acari: Ixodidae) collected at a focus in Alabama. J. Med. Entomol., 28:652–657.

LUCKHART, S., G. R. MULLEN, L. A. DURDEN, AND J. C. WRIGHT. 1992. *Borrelia* sp. in ticks recovered from white-tailed deer in Alabama. J. Wildl. Dis., 28:449–452.

MAGNARELLA, L., A. DENICOLA, K. STAFFORD, AND J. ANDERSON. 1995. *Borrelia burgdorferi* in an urban environment: white-tailed deer with infected ticks and antibodies. J. Clin. Microbiol., 33:541–544.

MOYER, P. L., A. S. VARELA, M. P. LUTTRELL, V. A. MOORE, D. E. STALLKNECT, AND S. E. LITTLE. 2006. White-tailed deer (*Odocoileus virginianus*) develop spirochetemia following experimental infection with *Borrelia lonestari*. Vet. Microbiol., 115:229–236.

RE, V., J. OCCI, AND R. MACGREGOR. 2004. Identifying the vector of Lyme disease. Am. Fam. Phys., 69:1935–1938.

SOARES, C. A., N. S. ZEIDNER, C. B. BEARD, M. C. DOLAN, G. DIETRICH, AND J. PIESMAN. 2006. Kinetics of *Borrelia burgdorferi* infection in larvae of refractory and competent tick vectors. J. Med. Entomol., 43:61–67.

STROMDAHL, E. Y., S. R. EVANS, J. J. O'BRIEN, AND A. G. GUTIERREZ. 2001. Prevalence of infection in ticks submitted to the human tick test kit program of the US Army Center for Health Promotion and Preventive Medicine. J. Med. Entomol., 38:67–74.

TAFT, S. C., M. K. MILLER, AND S. M. WRIGHT. 2005. Distribution of *Borreliae* among ticks collected from eastern states. Vector-Borne Zoonotic Dis., 5:383–389.

TENNESSEE WILDLIFE RESOURCES AGENCY. 2007. Cheatham Wildlife Management Area. Available via the Internet (http://www.state.tn.us/twra/gis/wmapdf/Cheathambw.pdf).

WILDLIFE CONTROL. 2006. Wildlife Control Deer Fact Sheet. Available via the Internet (http://wildlifecontrol.info/ccewdmp/Publications/Deer_factsheet.pdf).