NATURAL AND EXPERIMENTAL INFECTION OF WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) FROM THE UNITED STATES WITH AN EHRLICHIA SP. CLOSELY RELATED TO EHRLICHIA RUMINANTIUM

Authors: Yabsley, Michael J., Loftis, Amanda D., and Little, Susan E.

Source: Journal of Wildlife Diseases, 44(2) : 381-387

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-44.2.381

Journal of Wildlife Diseases, 44(2), 2008, pp. 381–387

NATURAL AND EXPERIMENTAL INFECTION OF WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) FROM THE UNITED STATES WITH AN *EHRLICHIA* SP. CLOSELY RELATED TO *EHRLICHIA RUMINANTIUM*

Michael J. Yabsley,[1,2,5] Amanda D. Loftis,[3] and Susan E. Little[4]

[1] Daniel B. Warnell School of Forestry and Natural Resources, University of Georgia, Athens, Georgia 30602, USA
[2] Southeastern Cooperative Wildlife Disease Study, Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602, USA
[3] Viral and Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, USA
[4] Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, Oklahoma 74078, USA
[5] Corresponding author (email: myabsley@uga.edu)

ABSTRACT: An *Ehrlichia* sp. (Panola Mountain [PM] *Ehrlichia* sp.) closely related to *Ehrlichia ruminantium* was recently detected in a domestic goat experimentally infested with lone star ticks (LSTs, *Amblyomma americanum*) collected from Georgia, USA. The infected goat exhibited pyrexia and mild clinical pathologic abnormalities consistent with ehrlichiosis. At least two other *Ehrlichia* species (*Ehrlichia chaffeensis* and *Ehrlichia ewingii*) are maintained in nature by a cycle involving LSTs as the primary vector and white-tailed deer (*Odocoileus virginanus*) as a known or suspected reservoir. To investigate the possibility that white-tailed deer are potential hosts of the PM *Ehrlichia* sp., whole blood samples collected from 87 wild deer from 2000 to 2002 were screened with a species-specific nested PCR assay targeting the citrate synthase gene. In addition, two laboratory-raised white-tailed deer fawns were each infested with 120 wild-caught LST adults from Missouri, USA, and blood samples were periodically collected and tested for the PM *Ehrlichia* sp. Of 87 deer tested from 20 locations in the southeastern United States, three (3%) deer from Arkansas, North Carolina, and Virginia were positive for the PM *Ehrlichia* sp. Wild-caught ticks transmitted the PM *Ehrlichia* sp. to one of two deer fawns, and colony-reared nymphal LSTs acquired the organism from the deer, maintained it transstadially as they molted to adults, and transmitted the PM *Ehrlichia* sp. to two naïve fawns. These findings indicate that white-tailed deer are naturally and experimentally susceptible to infection with an *Ehrlichia* sp. closely related to *E. ruminantium* and are able to serve as a source of infection to LSTs.

Key words: *Amblyomma*, cervid, *Cowdria*, *Ehrlichia chaffeensis*, *Ehrlichia ruminantium*, heartwater, lone star tick.

INTRODUCTION

Ehrlichia spp. are a group of tick-transmitted, intracellular, Gram-negative bacteria that cause disease in a wide range of hosts, including humans, domestic dogs, ruminants, equids, and felids (Rikihisa, 1991). *Ehrlichia chaffeensis*, the causative agent of human monocytic ehrlichiosis, is maintained in a cycle involving white-tailed deer (WTD; *Odocoileus virginianus*) as a primary reservoir and the lone star tick (LST; *Amblyomma americanum*) as a primary vector (Ewing et al., 1995; Lockhart et al., 1997a, b; Yabsley et al., 2003). Field evidence and experimental infection trials also suggest that WTD are important hosts for at least two other LST-vectored organisms, *Ehrlichia ewingii* and *Borrelia. lonestari*, and natural infections of WTD with all three organisms have been reported in much of the range of the LST (Lockhart et al., 1997a; Yabsley et al., 2002; Arens et al., 2003; Moore et al., 2003; Moyer et al., 2006).

Ehrlichia ruminantium, previously *Cowdria ruminantium*, the causative agent of heartwater (cowdriosis) in ruminants, is widely distributed in sub-Saharan Africa and is established on some islands in the Caribbean (Deem, 1998). Numerous species of *Amblyomma* ticks can transmit *E. ruminantium*, but *Amblyomma variegatum* and *Amblyomma hebraeum* are the two primary vectors in Africa. There is great concern that, were *E. ruminantium* introduced into the United States, the organism could readily establish in wildlife reservoirs and native ticks. White-tailed deer are experimentally

TABLE 1. Polymerase chain reaction (PCR) results for PM *Ehrlichia* sp. in 87 white-tailed deer (*Odocoileus virginianus*) collected from 20 locations in the southeastern United States.

Location[a]	Coordinates	County, state	PM *Ehrlichia* sp. no. positive/no. tested (%)
Pea Ridge NMP	36°26′58″N, 94°06′48″W	Benton, AR	1/4 (25)
Roanoke River NWR	35°59′37″N, 76°56′33″W	Bertie, NC	0/8
Unknown	37°26′15″N, 78°34′15″W	Buckingham, VA	0/1
Lakens Island	34°53′14″N, 76°35′39″W	Carteret, NC	0/7
Unknown	36°19′30″N, 79°22′45″W	Caswell, NC	0/1
Okefenokee NWR	30°44′35″N, 82°34′37″W	Charlton/Ware, GA	0/10
Unknown	37°26′38″N, 78°22′22″W	Cumberland, VA	1/1 (100)
Cape Hatteras NS	35°15′10″N, 75°35′03″W	Dare, NC	0/3
Unknown	36°11′27″N, 78°41′59″W	Granville, NC	0/1
Scatter Creek WMA	36°11′58″N, 90°32′36″W	Greene, AR	0/5
Mattamukseet NWR	35°27′24″N, 76°11′40″W	Hyde, NC	1/4 (25)
Piedmont NWR	33°10′55″N, 83°38′31″W	Jones, GA	0/5
West Kentucky WMA	37°08′00″N, 88°49′00″W	McCracken, KY	0/5
Elk City WMA	37°12′21″N, 95°47′59″W	Montgomery, KS	0/7
White Oak Plantation	30°43′33″N, 81°42′31″W	Nassau, FL	0/3
B.F. Grant Memorial Forest	33°22′34″N, 83°28′42″W	Putnam, GA	0/2
Big Hammock WMA	31°55′36″N, 81°56′10″W	Tattnall, GA	0/6
New Hill area	35°38′25″N, 78°56′52″W	Wake, NC	0/2
St. Marks NWR	30°09′01″N, 84°08′56″W	Wakulla, FL	0/10
Panther Swamp NWR	32°46′15″N, 90°32′56″W	Yazoo, MS	0/2
Total			3/87 (3.5%)

[a] NWR = National Wildlife Refuge; NMP = National Military Park; WMA = Wildlife Management Area; CC = Conservation Center; NS = National Seashore.

susceptible to infection with *E. ruminantium* (Dardiri et al., 1987), and the Gulf Coast, USA, tick, *Amblyomma maculatum*, has been experimentally shown to be a competent vector (Mahan et al., 2000). In addition, *E. ruminantium* has recently been recognized as a zoonotic disease in South Africa (Allsopp et al., 2005).

Recently an *Ehrlichia* sp. (Panola Mountain [PM] *Ehrlichia* sp.) closely related to *E. ruminantium* was detected in LSTs from Panola Mountain State Park in Georgia, USA (Loftis et al., 2006). The organism was detected in a domestic goat that was experimentally infested with wild-caught LSTs. This goat developed pyrexia and clinical pathologic changes consistent with ehrlichiosis (monocytosis, neutropenia with increased lymphocytes, decreased alkaline phosphatase activity). The DNA of the PM *Ehrlichia* sp. was detected in blood samples collected on days post–tick exposure (DPTE) 19, 21, and 34. Antibodies cross-reactive with *E. chaffeensis* were detected in serum samples, with a maximum titer of 256 on DPTE 38. Laboratory-raised LST nymphs acquired the PM *Ehrlichia* sp. from the goat and transstadially maintained the infection (Loftis et al., 2006). Recently the PM *Ehrlichia* sp. was detected via polymerase chain reaction (PCR) in a blood sample from a human patient from Atlanta, Georgia, with a history of a LST bite, fever, and muscle pain that resolved after treatment with doxycycline (Reeves et al., unpubl. data).

Because the PM *Ehrlichia* sp. is suspected to be transmitted by LSTs, and because WTD are susceptible to infection with three other LST-vectored organisms (Lockhart et al., 1997a, b; Yabsley et al., 2002; Moore et al., 2003; Yabsley et al., 2003), we hypothesized that WTD might be natural hosts for the PM *Ehrlichia* sp. In this study, we conducted a PCR-based survey of WTD blood samples collected

from WTD populations with known exposure to LST-vectored organisms. In addition, we infected WTD with the PM *Ehrlichia* sp. by transmission-feeding wild-caught LSTs from Missouri, USA, on two laboratory-raised WTD fawns, and we evaluated their ability to infect colony-reared nymphal LSTs.

MATERIALS AND METHODS

From 2000 to 2002, whole blood samples from 87 deer from 20 sites (Table 1) in the southeastern United States with known exposure to *A. americanum*–transmitted organisms were collected in Vacutainer EDTA tubes and frozen at −20 C until PCR testing. For PCR, DNA was extracted from 200 µl of whole blood using the GFX Genomic Blood Purification kit (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA) according to the manufacturer's instructions. To detect the PM *Ehrlichia* sp., a nested PCR protocol for the citrate synthase gene (~433 bp) (Loftis et al., 2008) was conducted using the external primers Ehr3CS-185F (5′-GCCACCGCAGATAGTTAGGGA) and Ehr3CS-777R (5′-TTCGTGCTCGTGGATCATAGTTTT) in a 25 µl reaction that contained 11 µl molecular biology-grade water, 2.5 µl 25 mM $MgCl_2$, 5 µl 5X colorless buffer (Promega, Madison, Wisconsin, USA), 0.25 µl 20 mM dNTPs (Promega), 0.5 µl of each primer (50 µM), 0.25 µl GoTaq® Flexi polymerase (Promega), and 5 µl of sample DNA. For the nested PCR, 1 µl of primary product was used as template in a 25 µl reaction containing the same PCR components except primers Ehr3CS-214F (5′-TGTCATTTCCACAGCATTCTCATC) and Ehr3CS-619R (5′-TGAGCTGGTCCCCACAAAGTT) (Loftis et al., 2008). Cycling conditions in both the primary and secondary reactions were 40 cycles of 94 C for 30 sec, 55 C for 30 sec, and 72 C for 30 sec. This protocol has a sensitivity of 10 gene copies as determined by PCR with a cloned plasmid containing the *gltA* gene from the *Ehrlichia* sp. (Loftis et al., 2008).

Assays of DNA from *E. chaffeensis*, *E. ewingii*, *Ehrlichia canis*, *Ehrlichia* sp. of raccoons, *Anaplasma phagocytophilum*, *Anaplasma platys*, and *Anaplasma* sp. of WTD with this PCR protocol were uniformly negative. DNA from a naturally infected *A. americanum* was used as a positive control. Stringent protocols and controls were utilized in all PCR assays to prevent and detect contamination. DNA extraction, primary amplification, secondary amplification, and product analysis were performed in separate dedicated laboratory areas. A negative water control was included in each set of DNA extractions, and one water control was included in each set of primary and secondary PCR reactions.

Two 6-mo-old, laboratory-reared white-tailed deer fawns (ID nos. 12 and 18) were housed in a tick-proof facility for the duration of these experiments. Before experimental exposure to *A. americanum*, both fawns were negative for antibodies reactive to *E. chaffeensis* and PCR-negative for *E. chaffeensis*, *E. ewingii*, the PM *Ehrlichia* sp., *A. phagocytophilum*, the *Anaplasma* sp. of WTD, and *Borrelia* spp. Adult *A. americanum* wild-caught in Missouri ($n=120$ per fawn) were placed in tick chambers secured in a mid-lateral position on each deer fawn. Whole blood samples were collected on DPTE 3, 7, 14, 24, 27, 29, 31, 34, 42, 49, and 56 and tested for DNA of the PM *Ehrlichia* sp. by PCR as described above. At DPTE 27, laboratory-raised *A. americanum* nymphs from Oklahoma State University ($n=200$ per deer) were fed on fawns 12 and 18. Replete nymphs from both deer fawns were collected, pooled, allowed to molt, and a subset of resulting adults ($n=20$) was individually dissected, all internal organs were removed and digested overnight in SDS/proteinase K, and nucleic acid was isolated by standard phenol/chloroform extraction followed by ethanol precipitation (Sambrook et al., 1989). Water controls were included during each extraction process. Resultant pellets were dissolved in 100 µl of molecular biology-grade water, and 5 µl were used in nested PCR for the PM *Ehrlichia* sp. as previously described. At necropsy, samples of skin, inguinal lymph node, mesenteric lymph node, spleen, kidney, bone marrow, lung, liver, and ear were collected from both deer and processed for routine histopathology and PCR testing for the PM *Ehrlichia* sp. as described above. For DNA extraction, tissues (~5 mg) were digested with proteinase K and extracted using the GFX extraction kit.

Remaining molted adult ticks that were acquisition fed as nymphs ($n=120$) were divided randomly into two pools of 60 ticks each and allowed to feed on two naïve deer fawns (ID numbers 8 and 32). Blood samples were collected from each deer every 3–4 days for 56 days post-transmission feeding and evaluated for evidence of infection. At necropsy, the same organs, plus the brain, were collected and processed as described for fawns 12 and 18.

RESULTS

The PM *Ehrlichia* sp. was detected in three of 87 (3%) deer from the southeastern United States by nested PCR (Table 1). A single 1-yr-old deer was coinfected with the PM *Ehrlichia* sp. and *E. ewingii*, and the two remaining PM *Ehrlichia*-positive deer (both 1.5-yr-old) were coinfected with *E. chaffeensis*. Sequence analysis of *gltA* amplicons from two deer were 100% identical to the PM *Ehrlichia* sp. detected in an experimentally infected goat (GenBank DQ363995) and from *A. americanum* from numerous southeastern states (Loftis et al., 2008). One *gltA* amplicon from a wild deer had a single polymorphic base (nucleotide 289 AR as numbered by DQ363995).

One of the two deer fawns (no. 18) experimentally infested with wild-caught adult *A. americanum* became PCR positive for the PM *Ehrlichia* sp. on DPTE 24 and was positive until DPTE 42. The *gltA* sequence of the PM *Ehrlichia* sp. from deer fawn 18 was 100% identical to GenBank no. DQ363995. The second fawn (no. 12) was PCR negative for the PM *Ehrlichia* sp. on all sampling dates. Eight of 20 (40%) adult LSTs that had been fed as nymphs on the two deer fawns were PCR positive for the PM *Ehrlichia* sp. Fawns 8 and 32, who each received 60 adult LSTs acquisition fed as nymphs, both became PCR positive for the PM *Ehrlichia* sp. by DPI 24 and 27, respectively, and one fawn remained PCR positive until DPI 52 (Table 2). At necropsy, samples of both of the lymph nodes that were examined, lung, and bone marrow were PCR positive for the PM *Ehrlichia* sp. (Table 2). No histopathologic lesions were noted in any deer fawns.

DISCUSSION

Our data provide the first evidence that deer are naturally infected with and experimentally susceptible to infection

TABLE 2. Polymerase chain reaction results for PM *Ehrlichia* sp. from blood samples and tissues collected at necropsy from four white-tailed deer fawns (*O. virginianus*) exposed to *Amblyomma americanum* adults.

	Days post–tick exposure (DPTE)																Tissues positive at necropsy[a]
	0	2 or 3	7	10	14	17	21	24	27	29	31	34 or 35	38	41 or 42	45	49	52	56	
Deer exposed to wild-caught ticks																			
Deer 12	—	—	—	nd[b]	—	nd	nd	—	—	—	—	—	nd	—	nd	—	nd	—	None
Deer 18	—	—	—	nd	—	nd	nd	+	+	+	+	+	nd	+	nd	—	nd	—	None
Deer exposed to experimental ticks																			
Deer 8	—	—	—	—	—	—	—	—	+	nd	nd	+	+	—	—	—	—	—	MLN, BM, LU
Deer 32	—	—	—	—	—	—	—	+	+	nd	+	+	+	+	+	+	+	—	ILN, MLN, BM

[a] ILN = inguinal; MLN = mesenteric lymph node; BM = bone marrow; LU = lung.

[b] nd = not done.

with the PM *Ehrlichia* sp., which is closely related to *E. ruminantium*. We also demonstrated that LSTs, the suspected vector of PM *Ehrlichia* sp., can transmit this agent to naïve deer fawns, and that LSTs can acquire infection from infected deer as nymphs and maintain that infection transstadially when they molt to adults; similar results were previously shown with LSTs fed on an infected goat (Loftis et al., 2006). This study further expands the known geographic range of the PM *Ehrlichia* sp. beyond Georgia to include parts of four additional US states (Arkansas, Missouri, North Carolina, and Virginia) and confirms the vector capacity of LSTs.

Because of the association of the PM *Ehrlichia* sp. and LSTs, we tested only deer populations with known exposure to LSTs and evidence of infection with other LST-vectored organisms. Coinfections of humans and domestic animals with multiple pathogens transmitted by the same vector are being increasingly recognized (Sexton et al., 1998; Kordick et al., 1999; Loebermann et al., 2006), as are coinfected individual ticks (Schulze et al., 2005; Mixson et al., 2006). These reports of coinfection resulted from infestations with single or limited numbers of ticks that are coinfected; because WTD are frequently infested with hundreds to thousands of LSTs, the likelihood of coinfection in wild WTD is increased (reported as high as 25%) (Yabsley et al., 2002, 2003; Arens et al., 2003; Moore et al., 2003). In the current study, all three wild deer that were positive for the PM *Ehrlichia* sp. also were infected with either *E. chaffeensis* or *E. ewingii*. Coinfection of individual WTD with *E. chaffeensis/E. ewingii*, *E. chaffeensis/B. lonestari*, and *E. ewingii/B. lonestari* has been detected in previous studies (Yabsley et al., 2002; Arens et al., 2003). Infestation of deer fawns with as few as 300 wild-caught *A. americanum* resulted in coinfection with *E. chaffeensis*, *E. ewingii*, and *B. lonestari* (Varela-Stokes, 2007). The majority of WTD that are PCR positive for *E. chaffeensis*, *E. ewingii*, and *B. lonestari* are ≤1.5 yr old (Lockhart et al., 1997b; Yabsley et al., 2002; Moore et al., 2003; Yabsley et al., 2003); a similar association with age was observed with the PM *Ehrlichia* sp.

WTD are highly susceptible to experimental infection with *E. ruminantium* and display significant morbidity and mortality (Dardiri et al., 1987). However, the PM *Ehrlichia* sp. did not cause mortality in the three experimentally infected deer. No health data were available for the three hunter-killed wild deer. Although one of our experimentally infected fawns did develop pyrexia, mild anemia, and depression concomitant with development of PM *Ehrlichia* sp. infection, simultaneous coinfection with a *Theileria* sp. and *E. chaffeensis* precluded accurate interpretation of serology or hemograms (Little et al., unpubl. data). Future work will be aimed at the in vitro isolation of the PM *Ehrlichia* sp. so that experimental infection and transmission trials can be conducted in monospecifically infected hosts.

Data from this study and others demonstrate that WTD are exposed to at least five ehrlichial species (*E. chaffeensis*, *E. ewingii*, PM *Ehrlichia* sp., *Anaplasma phagocytophilum*, and *Anaplasma* sp. of WTD) (Dawson et al., 1996; Belongia et al., 1997; Lockhart et al., 1997a; Yabsley et al., 2002, 2003; Arens et al., 2003; Moore et al., 2003). Four of these species are known to be zoonotic, and WTD have been shown to be competent reservoirs of *E. chaffeensis* and PM *Ehrlichia* sp. Because of the reported low level of serologic cross-reactivity between *E. chaffeensis* and the PM *Ehrlichia* sp. in goats (Loftis et al., 2006), it is possible that low-titer *E. chaffeensis* seroreactors in prior studies of WTD actually represent infection with the PM *Ehrlichia* sp, *E. chaffeensis*, *E. ewingii*, or mixed infections. The presence of multiple, serologically cross-reactive ehrlichiae also raises the possibility that prior infection of WTD

with *E. chaffeensis* or other ehrlichiae could confound serologic surveys for *E. ruminantium*, should it be introduced into the United States. Future studies should utilize an array of diagnostic assays for epidemiologic studies, and experimental infections should investigate coinfection dynamics.

ACKNOWLEDGMENTS

The authors thank M. Savage, S. Adams, D. Hurst, M. West, K. Allen, and the Animal Resources staff at Oklahoma State University for assistance with fawn care, and L. Parsons for excellent technical assistance. We also thank D. Osborne and personnel at GA DNR for assistance in obtaining fawns. This research was supported in part by 1R56AI062834-01A1 from the National Institutes of Health; Cooperative Agreement 2001-96130032-CA, Veterinary Services, APHIS, USDA; Cooperative Agreement 01ERAG0013, United States Geological Survey, Biological Resources Division, USDI; and sponsorship of SCWDS by the fish and wildlife agencies of Alabama, Arkansas, Florida, Georgia, Kentucky, Kansas, Louisiana, Maryland, Mississippi, Missouri, North Carolina, Ohio, Puerto Rico, South Carolina, Tennessee, Virginia, and West Virginia, USA. Support from the states to SCWDS was provided in part by the Federal Aid to Wildlife Restoration Act (50 Stat. 917).

LITERATURE CITED

Allsopp, M. T., M. Louw, and E. C. Meyer. 2005. *Ehrlichia ruminantium:* An emerging human pathogen. South African Medical Journal 95: 541.

Arens, M. Q., A. M. Liddell, G. Buening, M. Gaudreault-Keener, J. W. Sumner, J. A. Comer, R. S. Buller, and G. A. Storch. 2003. Detection of *Ehrlichia* spp. in the blood of wild white-tailed deer in Missouri by PCR assay and serologic analysis. Journal of Clinical Microbiology 41: 1263–1265.

Belongia, E. A., K. D. Reed, P. D. Mitchell, C. P. Kolbert, D. H. Persing, J. S. Gill, and J. J. Kazmierczak. 1997. Prevalence of granulocytic *Ehrlichia* infection among white-tailed deer in Wisconsin. Journal of Clinical Microbiology 35: 1465–1468.

Dardiri, A. H., L. L. Logan, and C. A. Mebus. 1987. Susceptibility of white-tailed deer to experimental heartwater infections. Journal of Wildlife Diseases 23: 215–219.

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 rDNA sequence from wild white-tailed deer (*Odocoileus virginianus*). Journal of Parasitology 82: 52–58.

Deem, S. L. 1998. A review of heartwater and the threat of introduction of *Cowdria ruminantium* and *Amblyomma* spp. ticks to the American mainland. Journal of Zoo and Wildlife Medicine 29: 109–113.

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.

Kordick, S. K., E. B. Breitschwerdt, B. C. Hegarty, K. L. Southwick, C. M. Colitz, S. I. Hancock, J. M. Bradley, R. Rumbough, J. T. McPherson, and J. N. MacCormack. 1999. Coinfection with multiple tick-borne pathogens in a Walker Hound kennel in North Carolina. Journal of Clinical Microbiology 37: 2631–2638.

Lockhart, J. M., W. R. Davidson, D. E. Stallknecht, J. E. Dawson, and E. W. Howerth. 1997a. Isolation of *Ehrlichia chaffeensis* from wild white-tailed deer (*Odocoileus virginianus*) confirms their role as natural reservoir hosts. Journal of Clinical Microbiology 35: 1681–1686.

———, ———, ———, ———, and S. E. Little. 1997b. Natural history of *Ehrlichia chaffeensis* (Rickettsiales: Ehrlichieae) in the Piedmont physiographic province of Georgia. Journal of Parasitology 83: 887–894.

Loebermann, M., V. Fingerle, M. Lademann, C. Fritzsche, and E. C. Reisinger. 2006. *Borrelia burgdorferi* and *Anaplasma phagocytophilum* coinfection. Emerging Infectious Diseases 12: 353–355.

Loftis, A. D., W. K. Reeves, J. P. Spurlock, S. M. Mahan, D. R. Troughton, G. A. Dash, and M. L. Levin. 2006. Infection of a goat with a tick-transmitted *Ehrlichia* from Georgia, U.S.A., that is closely related to *Ehrlichia ruminantium*. Journal of Vector Ecology 31: 213–222.

———, T. R. Mixson, E. Y. Stromdal, M. J. Yabsley, L. E. Garrison, P. C. Williamson, R. R. Fitak, P. E. Fuerst, D. J. Kelly, and K. W. Blount. 2008. Geographic distribution and genetic diversity of the *Ehrlichia* sp. from Panola Mountain in *Amblyoma americanum*. BMC Infectious Diseases 8: In press.

Mahan, S. M., T. F. Peter, B. H. Simbi, K. Kocan, E. Camus, A. F. Barbet, and M. J. Burridge. 2000. Comparison of efficacy of American and African *Amblyomma* ticks as vectors of heartwater (*Cowdria ruminantium*) infection by molecular

analyses and transmission trials. Journal of Parasitology 86: 44–49.

MIXSON, T. R., S. R. CAMPBELL, J. S. GILL, H. S. GINSBERG, M. V. REICHARD, T. L. SCHULZE, AND G. A. DASCH. 2006. Prevalence of *Ehrlichia*, *Borrelia*, and *Rickettsial* agents in *Amblyomma americanum* (Acari: Ixodidae) collected from nine states. Journal of Medical Entomology 43: 1261–1268.

MOORE, V. A., A. S. VARELA, M. J. YABSLEY, W. R. DAVIDSON, AND S. E. LITTLE. 2003. Detection of *Borrelia lonestari,* putative agent of southern tick-associated rash illness, in white-tailed deer (*Odocoileus virginianus*) from the southeastern United States. Journal of Clinical Microbiology 41: 424–427.

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

RIKIHISA, Y. 1991. The tribe Ehrlichiae and ehrlichial diseases. Clinical Microbiology Reviews 4: 286–308.

SAMBROOK, T. L., E. F. FRITSCH, AND T. MANIATIS. 1989. Molecular cloning: A laboratory manual. Cold Spring Harbor Press, Cold Spring Harbor, New York, 1,659 pp.

SCHULZE, T. L., R. A. JORDAN, C. J. SCHULZE, T. MIXSON, AND M. PAPERO. 2005. Relative encounter frequencies and prevalence of selected *Borrelia, Ehrlichia,* and *Anaplasma* infections in *Amblyomma* americanum and *Ixodes scapularis* (Acari:Ixodidae) ticks from central New Jersey. Journal of Medical Entomology 42: 450–456.

SEXTON, D. J., G. R. COREY, C. CARPENTER, L. Q. KONG, T. GANDHI, E. BREITSCHWERDT, B. HEGARTY, S. M. CHEN, H. M. FENG, X. J. YU, J. OLANO, D. H. WALKER, AND S. J. DUMLER. 1998. Dual infection with *Ehrlichia chaffeensis* and a spotted fever group rickettsia: A case report. Emerging Infectious Diseases 4: 311–316.

VARELA-STOKES, A. S. 2007. Transmission of bacterial agents from lone star ticks to white-tailed deer. Journal of Medical Entomology 44: 478–483.

YABSLEY, M. J., A. S. VARELA, C. M. TATE, V. G. DUGAN, D. E. STALLKNECHT, S. E. LITTLE, AND W. R. DAVIDSON. 2002. *Ehrlichia ewingii* infection in white-tailed deer (*Odocoileus virginianus*). Emerging Infectious Diseases 8: 668–671.

———, V. G. DUGAN, D. E. STALLKNECHT, S. E. LITTLE, J. M. LOCKHART, J. E. DAWSON, AND W. D. DAVIDSON. 2003. Evaluation of a prototype *Ehrlichia chaffeensis* surveillance system using white-tailed deer (*Odocoileus virginianus*) as natural sentinels. Vector Borne and Zoonotic Diseases 3: 195–207.

Received for publication 23 March 2007.