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PERSISTENT *EHRLICHIA CHAFFEENSIS* INFECTION IN WHITE-TAILED DEER

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ABSTRACT: Four white-tailed deer (*Odocoileus virginianus*) were inoculated intravenously with a deer-origin isolate (15B-WTD-GA) of *Ehrlichia chaffeensis*. The course of infection was monitored using indirect fluorescent antibody (IFA), polymerase chain reaction (PCR), and culture over a 9 m period. All deer became rickettsemic within 24 days post inoculation (DPI), and all developed antibody titers >1:64 to *E. chaffeensis* by 17 DPI. Titers in all deer fell below 1:64 during 87 to 143 DPI. One deer exhibited a second period of seropositivity (peak titer of 1:256) from 207 to 271 DPI but was culture and PCR negative during this period. Rickettsemia was confirmed by reisolation of *E. chaffeensis* as late as 73 to 108 DPI in three deer. Positive PCR results were obtained from femur bone marrow of one deer and from rumenal lymph node of another deer at 278 DPI. None of the deer developed clinical signs, hematologic abnormalities, or gross or microscopic lesions attributable to *E. chaffeensis*. Two uninoculated control deer were negative on all tests through 90 DPI at which time they were removed from the study. Herein we confirm that white-tailed deer become persistently infected with *E. chaffeensis*, have initial rickettsemias of several weeks duration and may experience recrudescence of rickettsemia, which reaffirm the importance of deer in the epidemiology of *E. chaffeensis*.

Key words: *Ehrlichia chaffeenis*, epidemiology experimental study, *Odocoileus virginianus*, white-tailed deer.

INTRODUCTION

Human monocytotropic ehrlichiosis (HME), a recently recognized tickborne zoonosis caused by *Ehrlichia chaffeensis*, has been reported primarily in the southeastern, south-central, and mid-Atlantic regions of the United States (McDade, 1990; Walker and Dumler, 1996). Current epidemiologic evidence indicates that the white-tailed deer (*Odocoileus virginianus*) is a major vertebrate reservoir for *E. chaffeensis*. High prevalences of *E. chaffeensis*-reactive antibodies have been detected in deer from many locations in the southeastern and south-central United States (Dawson et al., 1994a; Lockhart et al., 1995, 1996; Little et al., 1997, 1998), and deer have been experimentally infected with a human isolate (Arkansas strain) of *E. chaffeensis* (Dawson et al., 1994b; Ewing et al., 1995). Molecular evidence of natural *E. chaffeensis* infection has been obtained by polymerase chain reaction (PCR) testing of deer collected in Georgia, Missouri, and South Carolina (Little et al., 1997, 1998; Lockhart et al., 1997a). Proof that deer serve as a reservoir was obtained when *E. chaffeensis* was cultured from the blood of multiple animals from two of three seropositive deer populations in Georgia (Lockhart et al., 1997b).

White-tailed deer also are important hosts for all motile life stages of the lone-star tick (*Amblyomma americanum*) (Patrick and Hair 1978; Bloemer et al., 1986). The lone star tick initially was incriminated as a possible vector for *E. chaffeensis* based on detection of *E. chaffeensis* 16S rRNA from field-collected ticks (Anderson et al., 1992, 1993) and on a general spatial

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correlation between the distribution of HME cases and the geographic distribution of this tick (Eng et al., 1990; Dawson et al., 1994a). Later, temporal and site-specific spatial relationships were confirmed between the presence of *E. chaffeensis* antibodies and lone-star tick infestations among deer populations (Lockhart et al., 1995, 1996). Local lone star tick abundance also has been related to the prevalence of human cases (Standaert et al., 1995). Proof of vector status was provided by experimental transstadial transmission of *E. chaffeensis* from deer to deer by lone star ticks (Ewing et al., 1995).

Relatively little is known about the course, duration, or health consequences of *E. chaffeensis* infection in deer. A 1-mo-long study of two deer inoculated intravenously demonstrated seroconversion, confirmed transient rickettsemia, and disclosed *E. chaffeensis* antigen within prescapular, retropharyngeal, and mesenteric lymph nodes. Infected deer did not develop detectable evidence of clinical illness of specific pathologic changes (Dawson et al., 1994). Another short-term study of two needle-exposed deer produced similar results (Ewing et al., 1995). Two deer that were infected with *E. chaffeensis* through the bite of infected lone star ticks also seroconverted, had evidence (PCR assays) of intermittent rickettsemia, and did not develop clinical illness or pathologic changes specifically attributable to *E. chaffeensis* (Ewing et al., 1995). Among naturally infected deer, *E. chaffeensis* DNA has been detected in blood, lymph node, and spleen (Lockhart et al., 1997a, b; Little et al., 1997), and *E. chaffeensis* has been isolated from blood (Lockhart et al., 1997a) and lymph node (Little et al., 1998).

Persistent infection is characteristic of many species of *Ehrlichia*, and infection of at least several weeks duration has been described in laboratory mice and in humans infected with *E. chaffeensis* (Dumler et al., 1993; Telford and Dawson, 1996). However, other than the circumstantial evidence from naturally infected deer, there is no information on the ability of *E. chaffeensis* to establish a persistent infection in white-tailed deer. The purpose of this study was to evaluate the course of *E. chaffeensis* infection in white-tailed deer over a 9-mo period.

MATERIALS AND METHODS

Experimental design and sample collection

Six approximately 12-wk old, hand-reared white-tailed deer were acquired from the North Carolina Wildlife Resources Commission and moved to tick-free facilities (Department of Animal Resources, College of Veterinary Medicine, The University of Georgia, Athens, Georgia, USA). When the deer were acquired, indirect fluorescent antibody (IFA) testing of serum (Dawson et al., 1994a) and nested PCR testing of blood (Lockhart et al., 1997) from all deer produced negative results for evidence of infection by *E. chaffeensis*. Four deer were randomly selected to serve as principals, and two deer served as uninfected controls. Groups consisting of two principals and one control were co-housed in adjacent stalls. Deer were tranquilized by intramuscular injection of a mixture of 1.7 mg/kg of xylazine (Mobay Corporation, Shawnee, Kansas, USA) and 0.1 mg/kg ketamine (Fort Dodge Laboratories Inc., Fort Dodge, Iowa, USA) for experimental inoculations and blood sample collections and then reversed with 1.3 mg/kg of yohimbine (Lloyd Laboratory, Shenandoah, Iowa, USA) administered intravenously. Two principals (high-dose group) were inoculated intravenously with 3.7×10^6 *E. chaffeensis*-infected DH82 cells, and two principals (low dose group) were similarly inoculated with 2.5×10^4 infected cells. Two control deer were injected with 5.2×10^6 uninfected DH82 cells. The *E. chaffeensis* strain (15-WTD-B) used in this study was originally isolated in DH82 cells inoculated with blood from a wild naturally infected white-tailed deer from Clarke County, Georgia (Lockhart et al., 1997b).

For inoculation and blood sample collection, the lateroventral portion of the neck was shaved with electric clippers and the jugular groove was wiped with 95% ethanol. Blood samples were collected at Day 0 and at 10, 17, 24, 32, 45, 59, 73, 87, 108, 123, 143, 164, 187, 207, 227, 243, 248, 259, 271, and 278 days post-inoculation (DPI) from the principals. Control deer were sampled in the same way through DPI 90 (DPI 87 omitted) at which time they were humanely killed. Anticoagulated blood samples were collected in heparin for culture and in EDTA for PCR, light micros-

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copy, and hematology. Serum was collected for serologic testing. Deer were monitored at one- to four-day intervals for clinical signs. At necropsy, spleen and prescapular, rumenal, parotid, and iliac lymph nodes were collected aseptically and cultured as described by Lockhart et al. (1997b). Duplicates of these tissues plus bone (femur) marrow, heart, lung, liver, kidney, pancreas, skeletal muscle, brain, and bronchial and mesenteric lymph nodes were collected into 10% neutral buffered formalin for histopathologic examination. Tissues were processed for histopathologic study using standard procedures and stained using hematoxylin and eosin and Giemenez procedures. Portions of spleen, bone marrow, and prescapular, rumenal, parotid, and iliac lymph nodes were collected in separate plastic containers and processed for PCR assays as described by Lockhart et al. (1997b).

Serologic tests

Serum samples were tested for *E. chaffeensis*-reactive antibodies using the IFA assay as described by Dawson et al. (1994a). Samples were screened at a dilution of 1:64 in 0.01 M phosphate buffered saline (PBS) on spot slides of *E. chaffeensis*-infected DH82 cells. Samples giving positive results at 1:64 were further tested to determine titer endpoints using serial twofold dilutions. Samples nonreactive at 1:64 were considered negative (Lockhart et al., 1997a, b).

Isolation of organisms

In vitro isolation from blood followed the procedures of Dawson et al. (1994b) and Lockhart et al. (1997a) with slight modifications. Briefly, 10 ml of heparinized blood was transferred to sterile plastic tubes containing 25 ml of lysing buffer (150 mM NH4Cl, 0.7 mM KH2PO4, and 3 mM EDTA-Na2) and gently inverted. After 5 min at room temperature, the suspensions were centrifuged at 160 × *g* for 5 min and the supernatant fluid discarded; this procedure was repeated twice with the pellet following resuspension in lysing buffer. The pellet was resuspended in DH82 growth medium (minimum essential medium with 5% fetal bovine serum) and inoculated into a 25 cm^2 tissue culture flask containing uninfected DH82 cells. For 60 days post-inoculation (DPI), cultures were monitored weekly for evidence of infection using direct FA staining with fluorescein-labeled human anti-*E. chaffeensis* antibody as described by Dawson et al. (1994b).

Modifications of protocols of Dawson et al. (1994b) and Lockhart et al. (1997a) were adopted in order to control cell culture contamination by trypanosomes (*Trypanosoma cervi*), which had interfered with prior attempts to isolate *E. chaffeensis* from wild white-tailed deer (Lockhart et al., 1997b). These included replacement of media at 1–2 day intervals until trypanosomes could no longer be seen microscopically (40–100×) rather than at the predetermined twice per week intervals. In addition, all media was removed from inoculated flasks at 24 hr and centrifuged at 160 (*g* for 5 min at room temperature. The resulting. cell pellet was resuspended in 1 ml of fresh medium, sonicated in a water-cooled bell sonicator pulsed 90% of each sec for 15 sec at output control level 6 using a Branson Sonifier cell disrupter Model 450 (Branson, Danbury, Connecticut, USA), and the resulting sonicate returned to the culture falsk.

Molecular techniques

A portion of cells harvested from EDTA blood was processed for use in a nested PCR assay as described by Dawson et al. (1994b) with modifications. Briefly, lysing solution was mixed at a 1:100 ratio with EDTA whole blood. The mixture was incubated at room temperature for 5 min, centrifuged at 7,000 × *g* for 5 min, and the supernantant fluid discarded. An additional 1.5 ml of lysing solution was added to the pellet and the process repeated. Pelleted cells were washed once in 0.5 ml distilled water and centrifuged as above. The pellet was resuspended in 0.5 ml PBS, and DNA was extracted using Instagene Purification Matrix (BIO-RAD, Hercules, California, USA) as per manufacturer's directions. In the initial amplification, 10 μl of each sample was placed in a 100 μl reaction containing 10 mM of Tris-Cl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 5 μM Tetramethylammonium chloride (TMAC), 2.5 units Taq DNA polymerase (Promega, Madison, Wisconsin, USA), and 0.8 μM each of primer ECB (5′-CGTATTACCGCGGCTGCTGGCA-3′) and ECC (5′-AGAACGAACGCTGGCGGC-AAGCC-3′), which amplify a DNA fragment common among all known species of *Ehrlichia* and a few other bacterial species (Dawson et al., 1994b). The temperature profile for the initial amplification was 40 cycles of 1 min at 94 C, 2 min at 45 C, and 30 sec at 72 C. For the nested PCR amplification, 1 μl of the product from the first amlification was run in a 100 μl reaction as above using the primers HE1 (5′-CAATTGCTTATAACCTTTTGGTTATAAAT-3′) and HE3 (5′-TATAGGTACCGTCATTATCT-TCCCTAT-3′), which amplify DNA only from *E. chaffeensis* (Dawson et al., 1994b). The temperature

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profile for the nested reaction was the same as the first reaction except that the annealing temperature was 55 C and the extension time was 15 sec. Amplification products were electrophoresed in 1.5% agarose and were detected by staining with ethidium bromide and ultraviolet transillumination. Negative (water) and positive (*E. chaffeensis*-infected DH82 cells) controls were run with each series of PCR amplifications. In addition to standard precautionary procedures for PCR assays such as use of disposable gloves, bench top paper, plugged pipette tips, and biocontainment hoods with ultraviolet decontamination capabilities, each step in the procedure (DNA extraction, primary PCR reaction, secondary PCR reaction, and gel electrophoresis) was conducted in a separate laboratory.

Exposure of ticks

To evaluate whether deer remained infective to lone star ticks for an extended period post-inoculation, lone star tick nymphs were fed on two of the deer several months post-inoculation. The two deer suspected most likely to be rickettsemic were selected based on the frequency and duration of the earlier PCR assay and culture results. At DPI 243, 25 × 25 cm areas on the lateral thorax of deer 18 and 19 were shaved and the perimeters of 15 × 15 cm cloth patches with a center access slit with a velcro closure were glued to the skin using Livestock Identification Tag Cement (W.J. Ruscoe Company, Akron, Ohio, USA). Approximately 500 uninfected laboratory-reared *A. americanum* nymphs were introduced into each cloth container. On DPI 248, the cloth containers were removed and the engorged ticks (>95% of those placed on deer) were placed in a humidity chamber maintained at room temperature. Subsamples of engorged nymphs and molted adult ticks from deer 18 and 19 were processed and assayed using PCR as described by Lochart et al. (1997a). These PCR assays were conducted on both individual ticks and pools of ticks. Engorged nymphs were processed for PCR assay 2 days after they were removed from the deer. Molted adults were processed for PCR assay between 33 and 60 days after removal from the deer (3 pools at 42 days, 5 pools at 49 days, and 15 individuals at 63 days).

RESULTS

Serology, culture, and PCR

All four inoculated deer developed *E. chaffeensis* antibody titers >1:64 by 17 DPI (Table 1). Maximal titers were higher in the high-dose deer (1:128 and 1:1024) than in the low-dose deer (1:64). The durations of the initial antibody responses in high- and low-dose deer were similar, in each case persisting to 87 to 123 DPI. Following a seronegative period of at least 44 days, one-high dose deer developed a second period of seroreactivity that lasted 64 days and had a peak titer of 1:256. Control deer were seronegative (<1:64) at each of nine sampling dates from 0 to 90 DPI when sampling was discontinued.

Ehrlichia chaffeensis was reisolated on multiple occasions from the blood of three of the four inoculated deer (Table 1). Isolations were intermittently successful up to 73 DPI in the two high-dose deer and were consistently successful from one low-dose deer from 17 through 108 DPI, including five consecutive sampling dates from 45 to 108 DPI. Sixteen (3 to 6 per deer) of 78 (20%) cultures of blood were lost because of contamination.

Most cultures from tissues obtained at necropsy were lost to contamination within 32 days of incubation and therefore produced equivocal results. Reculture attempts using cells that had been suspended in cell culture medium and frozen in liquid nitrogen were all negative; however, the parotid lymph nodes of deer 15, 18, and 19 and iliac and rumenal lymph node from deer 15 were again contaminated upon reculture.

PCR assays infrequently detected *E. chaffeensis* DNA in the blood of three of four inoculated deer (Table 1). Two deer (one high- and one low-dose) were positive by PCR assay on two or more sampling dates from 10 to 32 DPI. Both low-dose deer had single positive PCR assays on blood well after inoculation (164 and 271 DPI).

PCR assays of brain, heart lung, liver, spleen, bone marrow, and prescapular, parotid, mesenteric, iliac, bronchial, and rumenal lymph nodes obtained at 278 DPI detected *E. chaffeensis* DNA in the bone marrow of one high-dose deer and in the rumenal lymph node of one low-dose deer.

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TABLE 1. Indirect fluorescent antibody (IFA) titers, polymerase chain reaction (PCR) results, and culture results for white-tailed deer experimentally infected with *Ehrlichia chaffeensis*.[a]

	Deer 14 (high dose)			Deer 18 (high dose)			Deer 15 (low dose)			Deer 19 (low dose)		
Day	IFA	PCR	Culture[b]	IFA	PCR	Culture	IFA	PCR	Culture	IFA	PCR	Culture
0	<64	−	−	<64	−	−	<64	−	−	<64	−	−
10	64	−	−	64	+	+	64	−	−	<64	−	−
17	128	−	Con	512	+	+	64	+	Con	64	−	+
24	64	−	+	512	+	Con	64	−	−	64	−	Con
32	64	−	−	1,024	−	−	64	+	−	64	−	−
45	128	−	−	1,024	−	+	64	−	−	64	−	+
59	128	−	−	512	−	−	64	−	−	64	−	+
73	64	−	+	512	−	+	64	−	−	64	−	+
87	64	−	−	256	−	−	<64	−	−	64	−	+
108	<64	−	−	128	−	−	64	−	−	<64	−	+
123	<64	−	Con	64	−	Con	<64	−	Con	<64	−	Con
143	<64	−	−	<64	−	−	<64	−	−	<64	−	Con
164	<64	−	−	<64	−	−	<64	−	−	<64	+	−
187	<64	−	−	<64	−	−	<64	−	−	<64	−	−
207	<64	−	Con	256	−	−	<64	−	−	<64	−	−
227	<64	−	Con	128	−	Con	<64	−	Con	<64	−	−
243	ND	ND	ND	64	−	−	ND	ND	ND	<64	−	−
248	<64	−	Con	ND	ND	ND	<64	−	−	ND	ND	ND
259	ND	ND	ND	64	−	−	ND	ND	ND	<64	−	−
271	<64	−	−	64	−	−	<64	+	−	<64	−	Con
278	64	−	Con	<64	−	Con	<64	−	−	<64	−	−
278Nx	NA	−	−	NA	+	−	NA	+	−	NA	−	−

[a] Two control deer (nos. 16 and 17) were negative on all tests through DPI 90 at which time they were removed from the study. ND = not done; Con = contaminated (inconclusive results); NA = not applicable; − = negative result; + = positive result.

[b] Culture results for blood except for last row. Entries for DPI 278Nx provide results of PCR assays and isolation attempts on tissues (see next) collected at necropsy on DPI 278; positive PCR assays were obtained only from bone marrow of deer 18 and rumenal lymph node of deer 15.

All blood samples (n = 9 each) and tissues (same as above) collected at 90 DPI from the two control deer were negative on all PCR assays.

Clinical evaluations, blood films, and histopathology

Clinical hematology conducted on principal and control deer through 45 DPI did not reveal any abnormalities attributable to *E. chaffeensis* infection (data not shown). Parameters monitored included white blood cell, erythrocyte, and platelet counts, differential white cell counts, hematocrit, mean corpuscular hemoglobin, and fibrinogen levels. Light microscopy of Giesma stained thin blood films through 45 DPI did not detect *E. chaffeensis* morulae in any deer; however, at each sampling period, all inoculated and control deer were infected with intraerythocytic organisms morphologically compatible with *Theileria cervi*. Deer 14 had circulating microfilariae morphologically compatible with those of the abdominal nematode *Setaria yehi*, and live and dead degenerating adult *S. yehi* were found in the abdominal cavity of this deer at necropsy.

Histopathologic examination of tissues collected at necropsy did not disclose any lesions specifically attributable to *E. chaffeensis*. Mild depletion of germinal centers was observed in the spleen in deer 14 and 15 and in mesenteric, prescapular, and/or iliac lymph nodes of deer 14, 15, 18, and 19. Scattered mild mineralization of renal collecting tubules was present in deer 14, 15, and 17, and mild chronic focal interstitial nephritis was noted in deer 15, 18, and 19. Mild diffuse hemosiderosis was

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found in the spleen of deer 17 and 19 and in the lung of deer 17. Deer 19 had multifocal granulomatous pneumonia of unknown origin.

Assays of ticks

PCR assays of 106 unfed *A. americanum* nymphs (pools of 5–20 individuals), to test for pre-existing infection in the laboratory colony, were negative. PCR assays of 50 freshly engorged nymphs (25 each from deer 18 and 19 in pools of 5) were negative. PCR assays of 110 molted adult ticks from deer 18 and deer 19 (8 pools of 5 ticks and 15 individual ticks) also were negative.

DISCUSSION

Because prior experimental *E. chaffeensis* infections in white-tailed deer were short-term and were conducted with a human isolate (Arkansas strain) (Dawson et al., 1994; Ewing et al., 1995), a comparison of the early course of infection with our 15B-WTD-GA isolate is of interest. Through 32 DPI response to infection with the 15B-WTD-GA strain of *E. chaffeensis* was similar to that of needle-exposed deer infected with the Arkansas strain (Dawson et al., 1994b; Ewing et al., 1995). In each study, seroconversion occurred at 10 to 17 DPI, rickettsemia usually was confirmed from 7 to 17 DPI but always by 24 DPI, and clinical illness was not observed. Deer infected with *E. chaffeensis* (Arkansas strain) via the bite of *A. americanum* ticks also became rickettsemic within 18 DPI and remained clinically normal but seroconverted later (at 25 to 28 DPI) than needle-exposed deer (Ewing et al., 1995). This difference may be related to route, dose, or both. Thus, all experimental infections in white-tailed deer have produced generally consistent results during the first month following exposure regardless of the strain of *E. chaffeensis* or the route of exposure utilized. Likewise, in the only other comparison of experimental exposure routes, Telford and Dawson (1996) found that the kinetics of antibody responses did not differ among needle-exposed mice inoculated with *E. chaffeensis* by intracardiac, intraperitoneal, or subcutaneous routes.

The initial serologic responses of deer in this study were transient and remained above the seropositve threshold of 1:64 only through 87 to 123 DPI. In the only other experimental infection study of deer longer than one month, Ewing et al. (1995) also noted transient, but shorter duration (33–36 day), antibody responses in deer infected with *E. chaffeensis* via bite of lone star ticks. The higher peak antibody titers in our high-dose deer suggests that the initial antibody responses may have been dose dependent; apparent dose dependent antibody responses to *E. chaffeensis* have been noted previously in needle-exposed C3H/HeJ mice (Lockhart and Davidson, 1999). A second distinct period of seroreactivity from 207 to 271 DPI in deer 18 suggests recrudescence of infection, although this was not verified by either isolation or PCR assays. Deer 14 also returned to a seropositive status (1:64) at the last sampling date, but interpretation of a single, low titer is problematic. Experimentally infected C3H/HeJ mice also had transient antibody responses with peak titers at 19 DPI, which then declined significantly by 103 DPI (Telford and Dawson, 1996); however, one mouse remained seropositive for well over a year.

The inability to detect *E. chaffeensis* in lone star ticks fed on two of the deer 8 months post-exposure agrees with PCR and cell culture assays, which failed to confirm that the deer were rickettsemic during the 5-day period of tick feeding. However, these findings must be interpreted cautiously because (1) the number of deer evaluated was small; (2) one deer not selected for tick feeding had a positive PCR assay from blood at termination of the study; (3) based on PCR assays, two of four deer may have had organisms sequestered in tissues at the end of the study; (4) the sensitivity of PCR and cell culture to detect rickettsemia in deer and

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the sensitivity of PCR to detect *E. chaffeensis* in ticks are unknown; and (5) the course of infection initiated by a single intravenous needle-inoculation may differ markedly from that of natural tick-induced infection. Furthermore, wild deer populations at locations where *A. americanum* is prevalent are almost certainly exposed to *E. chaffeensis*-infected ticks to at least some degree from March through October in most of the *E. chaffeensis* enzootic region of the United States. Thus, although the present study provides the first information on long-term *E. chaffeensis* infection in deer, from an epidemiologic standpoint, it likely is incomplete because it does not account for continuous exposure of wild deer to infected lone star ticks. Future experimental studies of the *E. chaffeensis* host/vector relationship between deer and ticks should be designed to include multiple or periodic tick-initiated exposures of deer to *E. chaffeensis* accompanied by multiple acquisition feeding attempts with uninfected ticks, which would more closely simulate natural circumstances. Future studies also should consider the possibilities of variation in individual animal response to exposure, intermittent or recrudescent rickettsemias, and sequestration of organisms, all of which are suggested from the present study. Finally, sensitivities of various diagnostic assays must be considered during interpretation of data during future studies.

Based on culture and PCR assays, two of four deer were frequently rickettsemic through 73 and 164 DPI; a third deer also was culture positive as late as 73 DPI. Rickettsemias of these durations are well beyond 40 DPI that previously was the longest documented rickettsemia in white-tailed deer (Ewing et al., 1995). The detection of *E. chaffeensis* DNA in lymph node and bone marrow of two deer at 278 DPI is evidence that deer may become persistently infected. Evidence of infection of reticuloendothelial tissues is consistent with an earlier experimental infection of deer where immunohistochemical staining detected *E. chaffeensis* within lymph node macrophages (Dawson et al., 1994b). It also is consistent with field studies in which up to 41% of the deer had *E. chaffeensis* DNA in lymph node or spleen (Lockhart et al., 1997a, b) and in which *E. chaffeensis* was isolated from the lymph node of a naturally infected deer (Little et al., 1998). Detection of *E. chaffeensis* DNA in blood of deer 15 at 271 DPI, 139 days after the last prior positive blood sample, is additional evidence that persistently infected deer may develop recrudescent rickettsemias and tends to corroborate the serologic evidence of recrudescence observed for deer 18. Collectively, these results suggest that deer may serve as a source of *E. chaffeensis* for ticks for months following an initial infection. This conclusion is consistent with data from field studies where 10 to 40% of the animals in seropositive deer populations are rickettsemic (Lockhart et al., 1997a, b; Little et al., 1997).

Chronic infection with persistent or recudescent rickettsemias is characteristic of vertebrate reservoirs of other species of *Ehrlichia* and is an important epidemiologic feature favoring transmission to tick vectors (Rikihisa, 1991). Lockhart et al. (1997a, b) proposed that *E. chaffeensis* is maintained in nature primarily by a tick vector-vertebrate reservoir system consisting of lone star ticks and white-tailed deer. The asserted role of deer was based on serologic, molecular, and culture confirmation that *E. chaffeensis* infection was prevalent among wild deer populations parasitized by lone star ticks (Dawson et al., 1994a; Lockhart et al., 1995, 1996, a, b; Little et al., 1997) and was supported by experimental infections that showed deer were suitable hosts capable of infecting lone star ticks (Dawson et al., 1994b; Ewing et al., 1995). The new findings that deer become persistently infected, that initial rickettsemias can last for months, and that persistently infected deer may develop recrudescent rickettsemias months later support the concept that deer are im-

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portant vertebrate reservoirs of *E. chaffeensis.*

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