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Isolation of *Ehrlichia chaffeensis* from Wild White-Tailed Deer (*Odocoileus virginianus*) Confirms Their Role as Natural Reservoir Hosts

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Field and experimental studies have implicated white-tailed deer (*Odocoileus virginianus*) as probable reservoir hosts for *Ehrlichia chaffeensis*, the causative agent of human monocytic ehrlichiosis, but natural infection in deer has not been confirmed through isolation of *E. chaffeensis*. Thirty-five white-tailed deer collected from three *Amblyomma americanum*-infested populations in Georgia were examined for evidence of *E. chaffeensis* infection by serologic, molecular, cell culture, and xenodiagnostic methods. Twenty-seven deer (77%) had *E. chaffeensis*-reactive indirect fluorescent-antibody assay titers of ≥1:64; and the blood, spleens, or lymph nodes of seven (20%) deer were positive in a nested PCR assay with *E. chaffeensis*-specific primers. *E. chaffeensis* was isolated in DH82 cell cultures from the blood of five (14%) deer, including two deer that were PCR negative. Combination of culture and PCR results indicated that six (17%) deer were probably rickettsemic and that nine (26%) were probably infected. Restriction digestion of PCR products amplified from deer tissues and cell culture isolates resulted in a banding pattern consistent with the *E. chaffeensis* 16S rRNA gene sequence. The sequences of all PCR products from deer tissues or cell culture isolates were identical to the sequence of the Arkansas type strain of *E. chaffeensis*. Xenodiagnosis with C3H mice inoculated intraperitoneally with deer blood, spleen, or lymph node suspensions was unsuccessful. When viewed in the context of previous studies, these findings provide strong evidence that *E. chaffeensis* is maintained in nature primarily by a tick vector-vertebrate reservoir system consisting of lone star ticks and white-tailed deer.

Human monocytic ehrlichiosis (HME), caused by *Ehrlichia chaffeensis*, has been diagnosed in 30 states and occurs mainly in the southeastern, south-central, and mid-Atlantic regions of the United States (9, 20, 25). Although HME is not a reportable disease, more than 400 cases have been confirmed since 1986, including occasional fatal infections (9, 13, 25).

There is convincing epidemiologic evidence that HME is a tick-borne zoonosis that is transmitted primarily by the lone star tick (*Amblyomma americanum*). The geographic distribution of HME cases conforms to the general distribution of *A. americanum* (9, 13), and PCR analyses have demonstrated *E. chaffeensis* DNA in adult *A. americanum* ticks from Arkansas, Georgia, Kentucky, Missouri, New Jersey, and North Carolina (2, 3, 19). In addition, *A. americanum* has been specifically identified as the source of tick bites among HME patients, including residents of a community with a high HME attack rate (24).

White-tailed deer (*Odocoileus virginianus*) have been implicated as a suspected reservoir host on the basis of both field and experimental studies. *E. chaffeensis*-reactive antibody titers of ≥1:128 were detected in 43% of 1,269 deer from 17 states. The prevalence of antibodies exceeded 70% among numerous deer populations in Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Maryland, Missouri, Mississippi, South Carolina, Texas, and Virginia (9). Experimentally inoculated white-tailed deer were rickettsemic for at least 2 weeks and developed *E. chaffeensis*-reactive antibodies, but did not become clinically ill (10, 14). Recently, PCR has been used to demonstrate that deer from some populations with a high prevalence of *E. chaffeensis*-reactive antibodies also have the *E. chaffeensis* 16S rRNA gene in their blood, spleens, or lymph nodes (16, 19).

White-tailed deer are well documented as important hosts for all three life stages of *A. americanum* (4, 5, 21), and as with patients with HME, the distribution of seropositive deer coincides with the general distribution of *A. americanum* (9). Field studies have demonstrated both temporal and site-specific geographic associations between *A. americanum* infestations and *E. chaffeensis*-reactive antibodies among wild deer populations (17, 18). Recent experimental studies have confirmed that *A. americanum* is capable of transstadial transmission of *E. chaffeensis* among white-tailed deer (14).

Despite the serologic, molecular, and ecologic evidence delineating the probable roles of lone star ticks and white-tailed deer in the epidemiology of HME presented above, *E. chaffeensis* has not been isolated from ticks or deer in nature. Serologic cross-reactions are known to occur among *Ehrlichia* species (23), and molecular identification of *E. chaffeensis* infection relies on comparison of nucleotide sequences with those from identified isolates, which in the case of *E. chaffeensis* consist of only three isolates from human patients (1, 6, 12). Because all field evidence of *E. chaffeensis* in deer has been based only on serology or on PCR (9, 11, 16–19) and because deer have recently been demonstrated to harbor a novel *Ehrlichia*-like organism which may produce serologic cross-reac-

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tion and can confound PCR assays (11, 16, 19), culture currently is the only method that can be used to determine definitively what species of *Ehrlichia* are present in deer. The objective of this study was to confirm by culture isolation that wild white-tailed deer are naturally infected with *E. chaffeensis.*

MATERIALS AND METHODS

Study sites and sample collection. This work was conducted at three sites within the piedmont physiographic region of Georgia, viz., Whitehall Experimental Forest (WHEF) in Clarke County; Cedar Creek Wildlife Management Area (CCWMA) in Jasper, Jones, and Putnam counties; and private property in Oglethorpe County. The three sites are within approximately 75 km of each other, and all were suspected of being sites where *E. chaffeensis* is endemic on the basis of previous serologic surveys of deer (9, 17–19). Five white-tailed deer were collected from one of the sites described above each month during July to September of 1995 and April to June of 1996.

Deer were collected by gunshot, and the ages of deer were determined by tooth replacement and wear (22). Each animal was examined visually for ticks as described by Lockhart et al. (17). Representative specimens of ticks present on the deer were collected in 70% ethanol and were submitted to the National Veterinary Services Laboratory (U.S. Department of Agriculture, Ames, Iowa) for identification. Immediately after death, the skin on the right thorax was cleansed with 95% ethanol, and cardiac blood samples were obtained in sterile heparinized, EDTA-containing, and nonheparinized containers and the containers were placed on ice. Both prescapular lymph nodes and a portion of spleen also were collected by aseptic techniques, placed in sterile containers, and chilled on ice. Processing of the samples for all diagnostic testing described below was conducted within 18 h.

Serologic testing. Serum harvested from clotted blood was tested for *E. chaffeensis*-reactive antibodies by the indirect fluorescent-antibody (IFA) assay described by Dawson et al. (9). Sera 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 a 1:64 dilution were tested to determine the titer endpoints by using serial twofold dilutions.

Isolation of organisms. Both cell culture and a xenodiagnostic procedure with C3H mice were used in attempts to isolate *E. chaffeensis*. In vitro isolation from blood followed the procedures of Dawson et al. (10), with slight modifications. Ten milliliters of heparinized blood was transferred to sterile plastic tubes containing 25 ml of lysing buffer (150 mM NH_4 Cl, 0.7 mM KH_2PO_4, 3 mM sodium EDTA), and the tubes were gently inverted. After 5 min at room temperature, the suspensions were centrifuged at $160 \times g$ for 5 min and the supernatant was discarded; this procedure was repeated twice with the pellet following resuspension in lysing buffer. The pellet was resuspended in DH82 cell growth medium (minimum essential medium with 5% fetal bovine serum) and inoculated into a 25-cm^2 tissue culture flask containing uninfected DH82 cells. Cells were harvested from the lymph nodes and spleens as described by Lockhart et al. (19). An approximately 3-mm cube of tissue from the interior of the organ was excised, placed in a sterile petri dish, minced, and then crushed with a sterile scalpel blade. Cells were suspended in 1 ml of PBS, and erythrocytes were lysed by the procedure described above. For 60 days postinoculation (DPI), cultures were monitored weekly for evidence of infection by direct fluorescent-antibody (FA) staining with fluorescein-labeled human anti-*E. chaffeensis* antibody as described by Dawson et al. (10).

Modifications of the protocols of Dawson et al. (10) and Lockhart et al. (19) were adopted in order to control cell culture contamination by trypanosomes (*Trypanosoma cervi*) that had interfered with prior attempts to isolate *E. chaffeensis* from wild white-tailed deer (19). These included replacement of media at 1- to 2-day intervals until trypanosomes were no longer visually present rather than at the predetermined intervals of twice per week. In addition, all medium was removed from inoculated flasks at 24 h and centrifuged. The resulting cell pellet was resuspended in 1 ml of fresh medium and sonicated in a water-cooled bell sonicator pulsed 90% of each second for 15 s at an output control level of 6 with a Branson Sonifier cell disrupter (model 450; Branson, Danbury, Conn.), and the resulting sonicate was returned to the culture flask.

Light and electron microscopy. If cell cultures exhibited positive FA reactions, a portion of the cells were harvested for light and electron microscopy. Light microscopy was performed with suspensions of DH82 cells scraped from flasks and spotted onto glass slides with a cytocentrifuge (Shandon Upshaw, Pittsburgh, Pa.). Slides were air dried and stained with Diff-Quik (Baxter Diagnostics, Inc., McGaw Park, Ill.) according to the manufacturer's directions. Stained slides were examined at ×200 to ×1,000 for ehrlichial organisms. For transmission electron microscopy, cells were scraped from a flask and pelleted by centrifugation at $200 \times g$ for 10 min. The resulting pellet was fixed in a mixture of 2% paraformaldehyde, 2% glutaraldehyde, and 0.2% picric acid in 0.1 M cacodylate buffer (pH 7.2 to 7.4) and then refixed in osmium tetroxide and dehydrated in graded alcohols. The pellet was embedded in Spurr epoxy resin, thin sectioned, stained with methanolic uranyl acetate and Reynold's lead citrate, and examined in a transmission electron microscope.

Xenodiagnostic assays. A second portion of heparinized blood, lymph node, and spleen from each deer was processed to obtain cells as described above. A 0.3-ml volume of each cell source preparation was injected intraperitoneally into a single C3H mouse (one mouse/tissue; three mice/deer). At approximately 15 and 30 DPI, 0.25-ml blood samples were obtained from the retroorbital sinus of each mouse, and serum was tested for *E. chaffeensis*-reactive antibodies by the IFA assay. For each monthly deer sampling, suspensions of uninfected and *E. chaffeensis*-infected DH82 cells harvested from a tissue culture flask were each inoculated into a C3H mouse as negative and positive controls, respectively.

Molecular diagnostic assays. A third portion of cells harvested from EDTA-treated blood, precapsular lymph node, and spleen from each deer were processed for use in a nested PCR assay as described by Dawson et al. (10), with modifications. Briefly, lysing solution was mixed at a 1:100 ratio with EDTA-treated whole blood or with cells harvested from lymph nodes and spleens that were adjusted to a concentration of approximately 3,000 cells per μl of PBS. The mixtures were incubated at room temperature for 5 min and centrifuged at $7,000 \times g$ for 5 min, and the supernatant was discarded. An additional 1.5 ml of lysing solution was added to the pellet and the process was repeated. Pelleted cells were washed once in 0.5 ml of distilled water and centrifuged as described above. The pellet was resuspended in 0.5 ml of PBS, and DNA was extracted by using the InstaGene Purification Matrix (Bio-Rad, Hercules, Calif.) according to the manufacturer's directions. In the initial amplification, 10 μl of each sample was placed in a 100-μl reaction mixture containing 10 mM Tris-Cl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM (each) deoxynucleoside triphosphate, 5 μM tetramethylammonium chloride, 2.5 U of *Taq* DNA polymerase (Promega, Madison, Wis.), and 0.8 μM (each) primer ECB (5′-CGTATTACCGCGGCTGCTGGC A-3′) and primer ECC (5′-AGAACGAACGCTGGCGGCAAGCC-3′). Primers ECB and ECC amplify a DNA fragment common among all known species of *Ehrlichia* and a few other bacterial species (10). The temperature profile for the initial amplification was 40 cycles of 1 min at 94°C, 2 min at 45°C, and 30 s at 72°C. For the nested PCR amplification, 1 μl of the product from the first amplification was run in a 100-μl reaction mixture as described above, but with primers HE1 (5′-CAATTGCTTATAACCTTTTGGTTATAAAT-3′) and HE3 (5′-TATAGGTACCGTCATTATCTTCCCTAT-3′). Primers HE1 and HE3 amplify DNA only from *E. chaffeensis* (10). The temperature profile for the nested reaction was the same as that for the first reaction, except that the annealing temperature was 55°C and the extension time was 15 s. Amplification products were electrophoresed in 1.5% agarose and were detected by staining with ethidium bromide and UV transillumination.

The identities of the PCR products were confirmed by both restriction fragment length polymorphism (RFLP) analysis and nucleotide sequencing. Restriction endonuclease digestion was performed with representative amplicons by using *Hae*III (Promega Corporation, Madison, Wis.). *Hae*III was selected because predicted restriction sites would produce 338- and 51-bp products (19), thereby distinguishing *E. chaffeensis* from an *Ehrlichia*-like organism commonly present among white-tailed deer (11, 16). The remaining PCR product was ethanol precipitated to concentrate DNA prior to restriction digestion. The PCR products were digested with *Hae*III for 75 min at 37°C, and digestion products were resolved on a 3% agarose gel stained with ethidium bromide and visualized by using UV transillumination. Fragment sizes were determined by comparison with PCR marker DNA (Promega Corporation). Both strands of DNA generated by PCR amplification from representative deer tissues and from all isolates obtained in cell culture were sequenced. Amplicons were purified with a Microcon spin filter (Amicon Incorporated, Beverly, Mass.) and were submitted with their corresponding primers to the Molecular Genetics Instrumentation Facility at The University of Georgia, Athens, for sequencing following the Applied Biosystems Incorporated protocol for the ABI 373A automated sequencer. The resulting sequences were aligned by using DNAsis Mac, version 2.0, and were compared to published sequence data for *E. chaffeensis.*

RESULTS

A total of 35 deer were examined, including 20 from WHEF, 10 from CCWMA, and 5 from the Oglethorpe County site. Seven deer were ≤6 months old, 17 were 7 to 18 months old, and 11 were ≥19 months old. Thirty-one (89%) deer had tick infestations, and *A. americanum* was by far the predominant species at all three sites (500 of 502 specimens identified). Single specimens of adult *Amblyomma maculatum* and adult *Ixodes scapularis* ticks were recovered from two deer at CCWMA. Seventy-seven percent of the deer had *E. chaffeensis*-reactive antibody titers of ≥1:64, with site-specific antibody prevalences of 65, 90, and 100% at WHEF, CCWMA, and the Oglethorpe County site, respectively (Table 1). If a more conservative titer of 1:128 was applied, the overall prevalence of antibodies was 46%, with corresponding site-specific prevalences of 35, 40, and 100%, respectively. The prevalence of antibody titers of ≥1:64 among various age classes of deer

TABLE 1. Summary of serologic tests, PCR assays, and cell culture attempts for *E. chaffeensis* in 35 white-tailed deer from three sites in Georgia

Site	Serologic tests			PCR assays (no. of deer positive/total no. tested)				Isolates obtained (no. of deer with isolates/total no. of deer tested (%)[b]		
	No. of deer with titers of ≥1:64/total no. of deer tested	Geometric mean titer[a]	Maximum titer	Blood	Lymph node	Spleen	Any tissue	Blood	Lymph node	Spleen
WHEF	13/20	115	1:512	3/20	1/20	1/20	4/20	3/10 (30)	0/11 (0)	0/13
CCWMA	9/10	94	1:256	1/10	1/10	2/10	3/10	2/10 (20)	0/9	0/10
Ogglethorpe County	5/5	194	1:512	0/5	0/5	0/5	0/5	0/1	0/4	0/5

[a] Data for samples negative at a 1:64 dilution were omitted.
[b] Data for cultures lost to contamination prior to at least 53 days were omitted.

were 57, 78, and 90% for animals that were ≤6, 7 to 18 months, and ≥19 months old, respectively.

The nested amplification with the *E. chaffeensis*-specific HE1 and HE3 primers produced a 389-bp product (Fig. 1) from 7 of 35 (20%) deer. Blood was positive for four (11%) of the animals, lymph nodes were positive for 2 (6%) of the animals, and spleens were positive for 3 (9%) of the animals (Table 1). Restriction digests of these PCR products consistently produced two fragments with the molecular weights predicted for the *E. chaffeensis* 16S rRNA gene sequence. Sequencing of selected PCR products disclosed that all were identical to the published sequence for the type strain of *E. chaffeensis*.

Ehrlichia chaffeensis was isolated from the blood of five deer in DH82 cell cultures, including two deer that were PCR negative. These five isolations represent a minimum 14% prevalence of rickettsemia, because some cultures were lost due to contamination before the end of the 60-day target period (Table 1). Isolates were identified on the basis of (i) the demonstration of organisms compatible with ehrlichiae in Diff-Quik-stained DH82 cells (Fig. 2), (ii) demonstration of ultrastructural features characteristic of ehrlichiae by electron microscopy (Fig. 3), (iii) positive reactions on direct FA tests with *E. chaffeensis* antiserum, (iv) generation of expected 389-bp PCR products from suspect DH82 cell cultures by using HE1 and HE3 primers in a nested design (Fig. 4), and (v) confirmation of PCR products as *E. chaffeensis* by RFLP analysis (Fig. 4) and nucleotide sequencing. All isolates had the predicted RFLP patterns, and all had nucleotide sequences identical to that of the type strain of *E. chaffeensis*.

Host characteristics and diagnostic findings for the five deer from which *E. chaffeensis* was isolated are presented in Table 2.

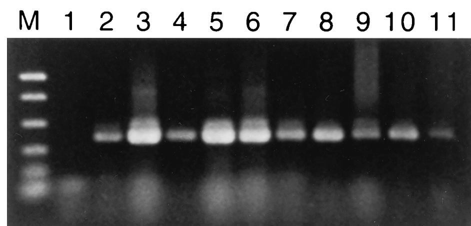

FIG. 1. Agarose gel electrophoresis of products amplified from white-tailed deer blood, lymph node, and spleen by a nested PCR with primers ECB and ECC as outside primers and primers HE1 and HE3 as inside primers. Lane 1, negative control; lane 2, positive control (tissue culture-maintained Arkansas strain of *E. chaffeensis*); lane 3, deer 14 spleen; lane 4, deer 15 blood; lane 5, deer 15 lymph node; lane 6, deer 17 blood; lane 7, deer 22 blood; lane 8, deer 28 spleen; lane 9, deer 30 lymph node; lane 10, deer 30 spleen; and lane 11, deer 32 blood; lane M, molecular size markers of 1,000, 750, 500, 300, 150, and 50 bp (Promega Corporation).

Ehrlichiae were first detected in cell cultures at 25 to 37 DPI. Cytopathic effects (CPEs), consisting mainly of detached cells in portions of the monolayer, were the first indication of positive cultures. When a CPE first appeared, direct FA testing sometimes produced only weak staining, but within 2 to 3 days staining became strongly positive. The rate of growth of the isolates was considerably slower than that of the Arkansas type strain, and in some cases, infection appeared cyclic, allowing for partial regrowth of the monolayer. The isolates grew more rapidly following passage to new flasks, resulting in infection of about 50% of the cells within 14 days. Although procedures to

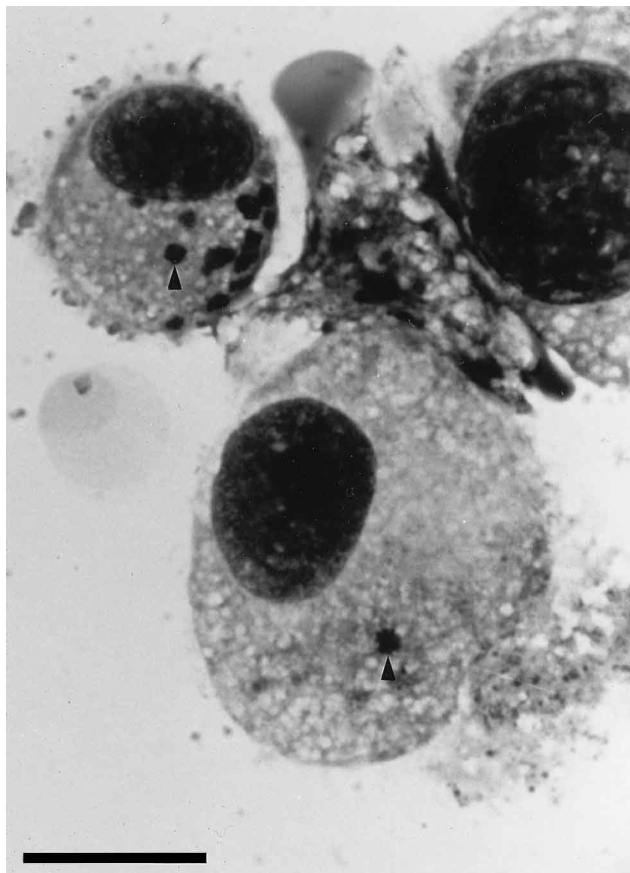

FIG. 2. Photomicrograph of morulae (arrowhead) of *E. chaffeensis* isolated from blood of white-tailed deer in the cytoplasm of DH82 cells. Diff-Quik stain was used. Bar, 15 μm.

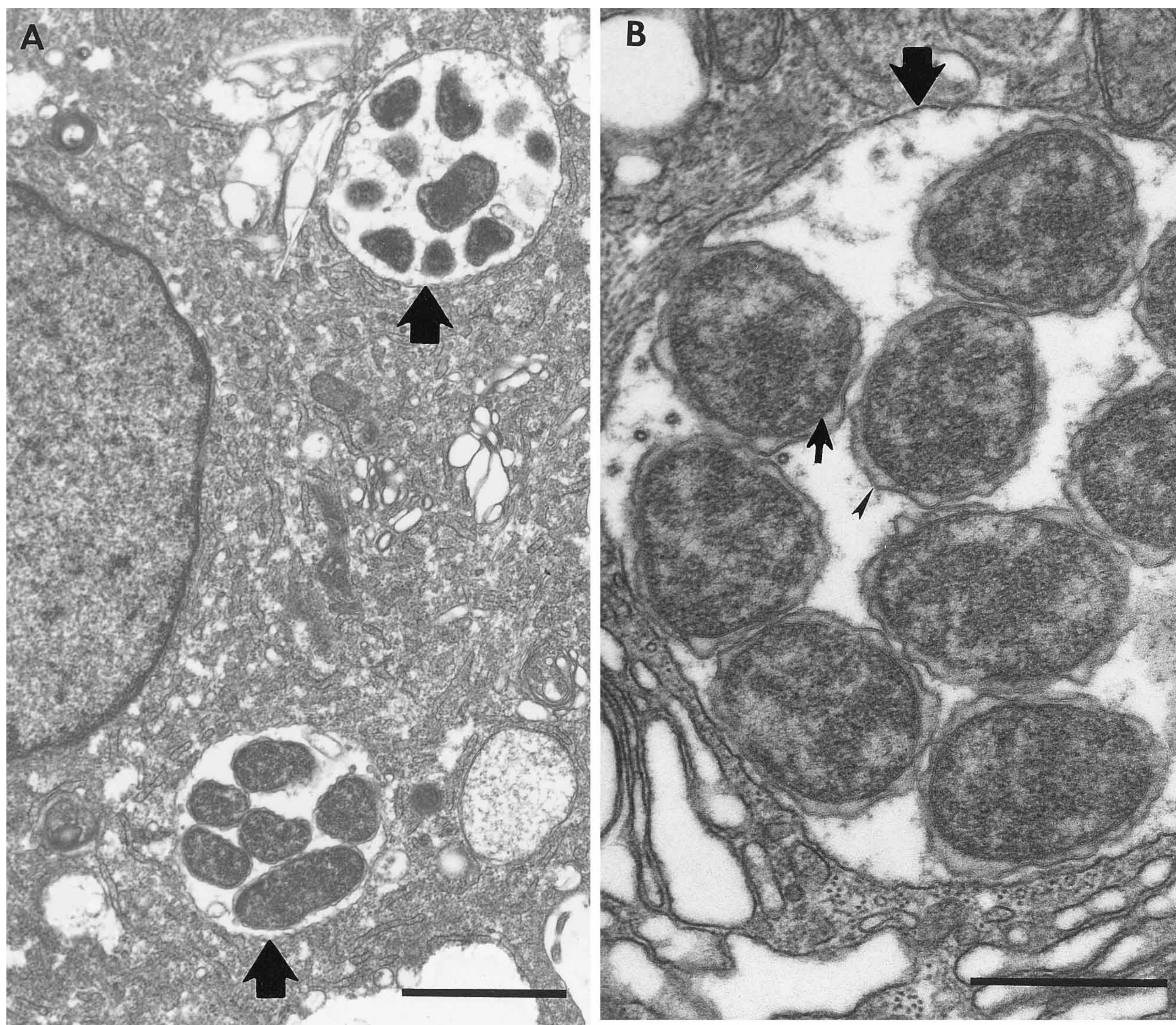

FIG. 3. Transmission electron micrographs of *E. chaffeensis* isolated from white-tailed deer blood in the cytoplasm of DH82 cells. (A) Organisms are seen in two membrane-bound morulae (arrows). Bar, 1.5 μm. (B) Higher magnification of a morula. A distinct membrane (large arrow) encloses multiple organisms that have an inner plasma membrane (small arrow) and an outer cell wall (arrowhead). Bar, 0.5 μm.

control trypanosomes were highly effective, numerous cultures were lost to bacterial contaminants prior to completion of the target 60-day culture period. Of 105 cultures, 67 were maintained for the full 60 days, 21 were maintained for 35 days, and 17 were maintained for less than 21 days.

None of the C3H mice injected with inocula prepared from blood, lymph nodes, or spleens developed *E. chaffeensis*-reactive antibodies, including those inoculated with tissues that were positive for *E. chaffeensis* by PCR or culture. All except one of the positive control mice developed titers of ≥1:64 by 15 DPI; one mouse had a titer of 1:32 at both 15 and 30 DPI. All negative control mice remained seronegative. Because none of the mice seroconverted, further testing of their tissues was not done.

DISCUSSION

The isolation of *E. chaffeensis* from naturally infected white-tailed deer represents the first recovery of this human pathogen from a nonhuman source. Detection of viable *E. chaffeensis* in the blood of 14% of the animals confirms that wild white-tailed deer are an epidemiologically important host capable of serving as a source of infection for tick vectors. On the basis of the results of PCR assays, additional deer likely were infected, and combination of culture and PCR results indicated an overall 26% prevalence of infection. Our data provide strong evidence that *E. chaffeensis* is maintained in nature primarily by a vector-reservoir host system consisting of lone star ticks and white-tailed deer. This position is corroborated by prior studies which have demonstrated that (i) experimentally infected white-tailed deer become rickettsemic, develop circulating antibodies, but remain clinically healthy (10, 14); (ii) many wild white-tailed deer populations have a high prevalence of both *E. chaffeensis*-reactive antibodies and the *E. chaffeensis* 16S rRNA gene in various tissues (9, 16–19); (iii) *A. americanum* is a competent vector capable of transstadial transmission of *E. chaffeensis* among white-tailed deer (14);

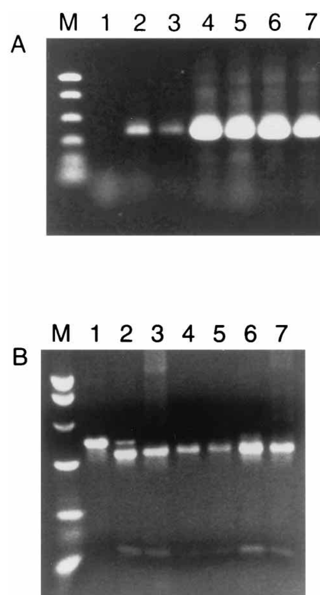

FIG. 4. PCR and restriction endonuclease digestion of five *E. chaffeensis* isolates obtained from the blood of naturally infected white-tailed deer. (A) Agarose gel electrophoresis of products amplified from isolates of *E. chaffeensis* by a nested PCR with primers ECB and ECC as outside primers and primers HE1 and HE3 as inside primers. Lane 1, negative control; lane 2, positive control (tissue culture-maintained Arkansas strain of *E. chaffeensis*), lanes 3 to 7, *E. chaffeensis* isolates from blood of deer 15, 16, 17, 22, and 23, respectively; lane M, molecular size markers of 1,000, 750, 500, 300, 150, and 50 bp (Promega Corporation). (B) Agarose gel electrophoresis of PCR products following restriction enzyme digestion with *Hae*III. Lane 1, positive control (undigested); lane 2, positive control (digested); lanes 3 to 7, digests of products generated from isolates obtained from the blood of deer 15, 16, 17, 22, and 23, respectively; lane M, molecular size markers of 1,000, 750, 500, 300, 150, and 50 bp (Promega Corporation). The top band in lane 2 is a residual undigested product.

(iv) the *E. chaffeensis* 16S rRNA gene is present among host-seeking adult *A. americanum* ticks at numerous locations (2, 3, 19); and (v) white-tailed deer serve as a major host for all mobile life stages of *A. americanum* (4, 5, 15, 21).

Although other species of ticks or mammals may be infected with *E. chaffeensis*, prior field studies at WHEF (19) and at an HME outbreak site in Tennessee (24) suggest that their epidemiologic importance probably is minimal compared to the epidemiologic importance of lone star ticks and white-tailed deer. This conclusion is supported by the general conformity between the geographic distributions of the vast majority of HME cases and lone star tick-infested white-tailed deer populations.

Both PCR and in vitro isolation were used successfully to detect infected deer; in contrast, use of C3H mice as a xenodiagnostic tool was unrewarding. A previous attempt to isolate *E. chaffeensis* from white-tailed deer at WHEF by using DH82 cell cultures was unsuccessful, with many cultures being lost to contamination by trypanosomes (19). While our modifications of the culture procedure greatly aided in the control of trypanosomes, its effect on culture sensitivity was not evaluated. However, the addition of a sonication step with return of the sonicate to the tissue culture flask may have enhanced isolation sensitivity either through the release of intracellular ehrlichiae or by increasing the duration of contact between the original inocula and DH82 cells. Alternatively, because monocytes are adherent, this procedure may not have affected *E. chaffeensis*-infected cells. An experimental evaluation of the benefit, if any, that sonication provides would be of value during future attempts to isolate *E. chaffeensis*.

The growth characteristics of the isolates in DH82 cells differed from those of the reference strain of *E. chaffeensis* by being slower to develop. The two original isolates of *E. chaffeensis* from humans had similar initial growth characteristics (8, 12). This may reflect an adaptation to DH82 cells by the reference strain or the effect of inoculating large numbers of organisms when DH82 cells are passaged. The CPE was similar to that produced by the reference strain.

Of the deer that were rickettsemic (determined by culture and PCR combined), all but one were yearling animals (10 to 16 months old) with antibody titers of ≤1:64, and all but one were collected during March to June, which is the period of peak host seeking by nymph and adult *A. americanum* ticks in the Georgia piedmont (7). The pathogenesis of *E. chaffeensis* infection in white-tailed deer is known only from relatively short-duration (23- to 61-day) experimental infections in four needle-exposed and two tick-infected deer. However, each of these six experimental animals developed a transient (≤30-day) rickettsemia, and in the case of deer infected by tick transmission, antibody titers declined to 1:64 within 2 months postexposure (10, 14). These findings suggest that yearling deer, whether they are naive animals or previously exposed animals in which circulating antibody levels have declined, may be especially important in the epizootiology of *E. chaffeensis*.

During recent field studies on *E. chaffeensis*, PCR testing disclosed a 16S rRNA gene of an undescribed *Ehrlichia*-like organism among many white-tailed deer populations (11, 16) and, at one location, in lone star ticks as well (19). This *Ehrlichia*-like organism, which is closely related to the *E. phagocytophila* genogroup (11), is important because it may confound serologic testing of deer or PCR-based testing of both deer and ticks (11, 16, 19). The isolation of *E. chaffeensis* leaves no doubt that *E. chaffeensis*-reactive antibodies among wild deer result from *E. chaffeensis* infection; however, this does not

TABLE 2. Date, location, host characteristics, and microbiologic results for *E. chaffeensis* isolates from white-tailed deer

Date (mo/day/yr)	Location	Deer			Titer	PCR results for the following[a]:			Day[b]
		Animal no.[c]	Age (mo)	Gender[d]		Blood	Lymph node	Spleen	
9/27/1995	WHEF	15	18	M	1:64	Pos	Pos	Neg	37
3/19/1996	WHEF	16	9	F	<1:64	Neg	Neg	Neg	37
3/19/1996	WHEF	17	9	M	<1:64	Pos	Neg	Neg	37
4/24/1996	CCWMA	22	11	F	1:64	Pos	Neg	Neg	25
4/24/1996	CCWMA	23	11	F	1:64	Neg	Neg	Neg	25

[a] Pos, positive; Neg, negative.
[b] The day that the isolate was first detected on the basis of the CPE and FA testing.
[c] Isolate identification numbers for deer 15, 16, 17, 22, and 23 were 15B-WTD-GA, 16B-WTD-GA, 17B-WTD-GA, 22B-WTD-GA, and 23B-WTD-GA, respectively.
[d] M, male; F, female.

exclude the possibility of serologic cross-reactions due to the *Ehrlichia*-like organism infecting deer. These diagnostic issues and the zoonotic potential of the *Ehrlichia*-like organism infecting deer merit further investigation.

White-tailed deer and the ticks that parasitize them should be a central focus of future epidemiologic studies of human ehrlichiosis. As has been recommended previously (9, 18), monitoring of deer for ehrlichial infections may be the most efficient means of delineating the geographic distribution of these pathogens and may be beneficial in estimating the risk of human exposure.

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REFERENCES

1. **Anderson, B. E., J. E. Dawson, D. C. Jones, and K. H. Wilson.** 1991. *Ehrlichia chaffeensis*, a new species associated with human ehrlichiosis. J. Clin. Microbiol. **29:**2838–2842.
2. **Anderson, B. E., J. W. Sumner, J. E. Dawson, T. Tzianabos, C. R. Greene, J. G. Olson, D. B. Fishbein, M. Olsen-Rasmussen, B. P. Holloway, E. H. George, and A. F. Azad.** 1992. Detection of the etiologic agent of human ehrlichiosis by polymerase chain reaction. J. Clin. Microbiol. **30:**775–780.
3. **Anderson, B. E., K. G. Sims, J. G. Olson, J. E. Childs, J. F. Piesman, C. M. Happ, G. O. Maupin, and B. J. B. Johnson.** 1993. *Amblyomma americanum*: a potential vector of human ehrlichiosis. Am. J. Trop. Med. Hyg. **49:**239–244.
4. **Bloemer, S. R., E. L. Snoddy, J. C. Cooney, and K. Fairbanks.** 1986. Influence of deer exclusion on populations of lone star ticks and American dog ticks (Acari:Ixodidae). J. Econ. Entomol. **79:**679–683.
5. **Bloemer, S. R., R. H. Zimmerman, and K. Fairbanks.** 1988. Abundance, attachment sites, and density estimators for lone star ticks (Acari:Ixodidae) infesting white-tailed deer. J. Med. Entomol. **25:**295–300.
6. **Chen, S., V. L. Popov, H. Feng, and D. H. Walker.** 1996. Isolation and analysis of a new strain of *Ehrlichia chaffeensis* (abstract). *In* Proceedings of the 12th Sesquiannual Meeting of the American Society for Rickettsiology and Rickettial Diseases.
7. **Davidson, W. R., D. A. Siefken, and L. H. Creekmore.** 1994. Seasonal and annual abundance of *Amblyomma americanum* (Acari:Ixodidae) in central Georgia. J. Med. Entomol. **31:**67–71.
8. **Dawson, J. E., B. E. Anderson, D. B. Fishbein, J. L. Sanchez, C. S. Goldsmith, K. H. Wilson, and C. W. Duntley.** 1991. Isolation and characterization of an *Ehrlichia* sp. from a patient diagnosed with human ehrlichiosis. J. Clin. Microbiol. **29:**2741–2745.
9. **Dawson, J. E., J. E. Childs, K. L. Biggie, C. Moore, D. Stallknecht, J. Shaddock, J. Bouseman, E. Hofmeister, and J. G. Olson.** 1994. White-tailed deer as a potential reservoir of *Ehrlichia* spp. J. Wildl. Dis. **30:**162–168.
10. **Dawson, J. E., D. E. Stallknecht, E. W. Howerth, C. Warner, K. Biggie, W. R. Davidson, J. M. Lockhart, V. F. Nettles, J. G. Olson, and J. E. Childs.** 1994. Susceptibility of white-tailed deer (*Odocoileus virginianus*) to infection with *Ehrlichia chaffeensis*, the etiologic agent of human ehrlichiosis. J. Clin. Microbiol. **32:**2725–2728.
11. **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*). J. Parasitol. **82:**52–58.
12. **Dumler, J. S., S. M. Chen, K. Asanovich, E. Trigiani, V. L. Popov, and D. H. Walker.** 1995. Isolation and characterization of a new strain of *Ehrlichia chaffeensis* from a patient with nearly fatal monocytic ehrlichiosis. J. Clin. Microbiol. **33:**647–655.
13. **Eng, T. R., J. R. Harkess, D. B. Fishbein, J. E. Dawson, C. N. Greene, M. A. Redus, and F. T. Satalowich.** 1990. Epidemiologic, clinical, and laboratory findings of human ehrlichiosis in the United States, 1988. JAMA **264:**2251–2258.
14. **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). J. Med. Entomol. **32:**368–374.
15. **Haile, D. G., and G. A. Mount.** 1987. Computer simulation of population dynamics of the lone star tick, *Amblyomma americanum* (Acari: Ixodidae). J. Med. Entomol. **24:**356–369.
16. **Little, S. E., J. E. Dawson, J. M. Lockhart, D. E. Stallknecht, C. K. Warner, and W. R. Davidson.** 1997. Development and use of specific polymerase reaction for the detection of an organism resembling *Ehrlichia* sp. in white-tailed deer. J. Wildl. Dis. **33:**246–253.
17. **Lockhart, J. M., W. R. Davidson, J. E. Dawson, and D. E. Stallknecht.** 1995. Temporal association of *Amblyomma americanum* with the presence of *Ehrlichia chaffeensis* reactive antibodies in white-tailed deer. J. Wildl. Dis. **31:**119–124.
18. **Lockhart, J. M., W. R. Davidson, D. E. Stallknecht, and J. E. Dawson.** 1996. Site-specific geographic association between *Amblyomma americanum* (Acari: Ixodidae) infestations and *Ehrlichia chaffeensis*-reactive (Rickettsiales: Ehrlichieae) antibodies in white-tailed deer. J. Med. Entomol. **33:**153–158.
19. **Lockhart, J. M., W. R. Davidson, D. E. Stallknecht, J. E. Dawson, and S. E. Little.** Natural history of *Ehrlichia chaffeensis* in the piedmont physiographic region of Georgia. J. Parasitol., in press.
20. **McDade, J. E.** 1990. Ehrlichiosis—a disease of animals and humans. J. Infect. Dis. **161:**609–617.
21. **Patrick, C. D., and J. A. Hair.** 1978. White-tailed deer utilization of three different habitats and its influence on lone star tick populations. J. Parasitol. **64:**1100–1106.
22. **Severinghaus, C. W.** 1949. Tooth development and wear as criteria of age in white-tailed deer. J. Wildl. Manage. **13:**195–216.
23. **Shankarappa, B., S. K. Dutta, and B. L. Mattingly-Napier.** 1992. Antigenic and genomic relatedness among *Ehrlichia risticii*, *Ehrlichia sennetsu*, and *Ehrlichia canis*. Int. J. Syst. Bacteriol. **42:**127–132.
24. **Standaert, S. M., J. E. Dawson, W. Schaffner, J. E. Childs, K. L. Biggie, J. Singleton, Jr., R. R. Gerhardt, M. L. Knight, and R. H. Hutcheson.** 1995. Ehrlichiosis in a golf-oriented retirement community. N. Engl. J. Med. **333:**420–425.
25. **Walker, D. H., and J. S. Dumler.** 1996. Emergence of the ehrlichioses as human health problems. Emerg. Infect. Dis. **2:**18–29.