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Detection of *Ehrlichia* spp. in the Blood of Wild White-Tailed Deer in Missouri by PCR Assay and Serologic Analysis

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Blood samples collected from wild deer in Missouri in November of 2000 and 2001 were positive by PCR assays for *Ehrlichia chaffeensis* (50 of 217; 23%), *Ehrlichia ewingii* (44 of 217; 20%), and *Anaplasma* species (214 of 217; 99%). Nucleotide sequences of selected amplicons from the assay for anaplasma matched sequences of the white-tailed deer agent. Serologic analysis of 112 deer sampled in 2000 showed a very high prevalence of antibodies to *E. chaffeensis* (97 of 112; 87%) and a low prevalence of antibodies reactive with *Anaplasma phagocytophila* (2 of 112; 2%).

Ehrlichioses are important emerging tick-borne infections of humans. Two species, *Ehrlichia chaffeensis* and *Anaplasma* (formerly *Ehrlichia*) *phagocytophila*, are responsible for most human ehrlichioses in the United States. Recently, we reported human infections with *Ehrlichia ewingii* (4), which was previously known as a cause of canine granulocytic ehrlichiosis (1, 5, 10, 12). Our laboratory has used molecular assays for the detection of ehrlichiae in human patients from St. Louis and the surrounding area since 1994. During that time, we detected *E. chaffeensis* in 90% of cases and *E. ewingii* in the remaining 10% of cases. We have not detected human infection with *A. phagocytophila* despite the use of a broad-range (PCR) assay with the ability to detect this organism.

White-tailed deer (*Odocoileus virginianus*) serve as a reservoir of *E. chaffeensis*, *A. phagocytophila*, and the *Anaplasma*-like white-tailed deer agent (WTD agent) (3, 7, 13, 14). To gain a further understanding of the natural history of *E. ewingii*, we undertook a study of *Ehrlichia* and *Anaplasma* species in which we collected and analyzed, by PCR and serologic assays, blood samples from deer killed in central Missouri during two consecutive hunting seasons.

Blood was collected from 112 wild deer that were killed during the firearm hunting season on 11 or 12 November 2000 and from 105 deer killed on 10 November 2001. Deer were killed and field dressed in Boone County, Mo. (in the central part of the state), and sampled at a Missouri Department of Conservation Wildlife Check Station near Columbia, Mo. The deer were estimated to be between 1 and 4 years old, and approximately 71% were males. Most of the deer had attached ticks present at the time of blood collection. Pooled blood within the chest cavity was collected with a sterile 12-ml syringe (Monoject; Sherwood Medical, St. Louis, Mo.). Care was taken not to cross-contaminate the specimens from different deer. Blood samples were maintained at 4°C until processed. DNA was extracted from 400 μl of whole blood by using the QIAamp Blood kit (Qiagen, Inc., Valencia, Calif.) and resuspended in 100 μl of 10 mM Tris-EDTA buffer (pH 9.0). Plasma was stored at −70°C until used for serologic testing.

The 16S rRNA gene (rDNA) PCR assays used in the survey were those previously described for testing of human specimens (4). A screening assay employed broad-range primers for detection of most *Ehrlichia* and *Anaplasma* species. Positive samples were retested by using separate assays designed to specifically target *E. chaffeensis*, *E. ewingii*, and *Anaplasma* species (4). An additional species-specific assay for *Ehrlichia canis* using a forward primer designated CAN (5′-CAATTAT TTATAGCCTCTGGCTATAGGA) was also included. Reaction mixtures were set up as described previously (4), and amplifications were performed in a DNA thermal cycler (model 480; Perkin-Elmer, Norwalk, Conn.).

The results of species-specific PCR assays are shown in Table 1. The prevalence ratios of *E. chaffeensis* and *E. ewingii* were similar for both years, although fewer deer were positive in 2001. The assay using the anaplasma primers yielded a positive result for 98.6% of the 217 deer sampled. Thirteen deer from 2000 and eight from 2001 were positive for all three agents (*E. chaffeensis*, *E. ewingii*, and *Anaplasma* spp.). The two deer from 2000 and the one from 2001 that were negative with the primers targeting *Anaplasma* species were also negative by the other PCR assays.

To confirm that the PCR assays had detected the target species, five randomly chosen specimens that were positive for *E. chaffeensis* and five that were positive for *E. ewingii* were retested with the species-specific assays and amplicons were sequenced with the downstream primer (HE3). The resulting sequences from deer that were PCR positive for *E. chaffeensis* and *E. ewingii* exactly matched our previous *E. chaffeensis* sequences and the sequence with GenBank no. U60476 and our previous *E. ewingii* sequences (4) and the sequence with GenBank no. U96436, respectively.

The large proportion of deer that were positive with the

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TABLE 1. Detection of *Ehrlichia* or *Anaplasma* bacteria by PCR assay

Yr (no. of deer)	No. (%) of deer infected			
	E. chaffeensis	*E. ewingii*	*E. canis*	*Anaplasma* spp.[a]
2000 (112)	29[b] (26)	31[b] (28)	0	110 (98)
2001 (105)	21[c] (20)	13[c] (12)	ND[d]	104 (99)

[a] Nucleotide sequences of select amplicons matched that of the WTD agent, an unclassified *Anaplasma*-like bacterium.
[b] Includes 13 deer that were positive for both *E. chaffeensis* and *E. ewingii.* All deer positive for *E. chaffeensis* and *E. ewingii* were also positive with the assay for *Anaplasma* species.
[c] Includes eight deer that were positive for both *E. chaffeensis* and *E. ewingii.*
[d] ND, not done.

anaplasma primers prompted us to investigate whether the assay had detected *A. phagocytophila* or the WTD agent, an *Anaplasma*-like bacterium that has not received a species designation. Comparisons of 16S rDNA sequences indicate that the WTD agent is most closely related to *Anaplasma platys* (9). The WTD agent has been found in as many as 65% of deer tested in other studies (3, 7). Amplicons from the broad-range assays of deer samples that were positive only with the anaplasma primers were sequenced. A GenBank search showed that the sequences were identical to a sequence deposited for the WTD agent (GenBank no. U27101). The sequences differed from *E. chaffeensis* and *E. ewingii* at five positions (83, 110, 120, 131, and 134) and from *A. phagocytophila* at three of the same five positions (positions 83, 120, and 131). *E. chaffeensis* and *E. ewingii* are nearly identical across this region (except at position 81), and they differ from *A. phagocytophila* at three positions (83, 110, and 134).

Because the 16S rDNA sequences of *A. phagocytophila* and the WTD agent are very similar, additional PCR testing and sequencing was performed on 10 samples that were positive only in the assay for *Anaplasma* species. Two assays, one that amplifies a segment of the *groESL* operon of *A. phagocytophila* (18, 19) and another that amplifies a segment of the 16S rDNA of *A. phagocytophila* (15), were used. The *groESL* PCR assay was performed because it does not detect the WTD agent (J. W. Sumner, unpublished data). All of the samples were negative, indicating that those deer were not infected with *A. phagocytophila*, although the samples were positive by the PCR targeting the 16S rDNA. The nested products from four samples were sequenced in both directions by using primers GE9 and GE2. The sequences, consisting of 496 bp near the 5′ end of the 16S rDNA, were identical to each other and to sequences deposited in the GenBank database for the WTD agent, with the exception of several ambiguity codes contained in the latter. The sequence differed from sequences deposited for *E. chaffeensis*, *E. ewingii*, and *A. phagocytophila* at 21, 22, and 7 positions, respectively.

Deer plasma was analyzed for the presence of antibodies reactive with *E. chaffeensis* or *A. phagocytophila* by a fluorescent-antibody technique previously described (6, 17). *E. chaffeensis* (Arkansas strain grown in DH82 canine macrophage cells) and *A. phagocytophila* (USG3 strain grown in HL-60 cells) antigens were obtained as frozen infected-cell suspensions containing dimethyl sulfoxide as a cryopreservative. A titer of ≥64 was considered to be positive.

Serologic testing data demonstrated an extremely high prevalence (97 of 112; 87%) of antibodies reactive in the *E. chaffeensis* assay and a very low prevalence (2 of 112; 2%) of antibodies reactive in the *A. phagocytophila* assay (Table 2). All 97 deer that were PCR positive for *E. chaffeensis* or *E. ewingii* had positive titers in tests using the *E. chaffeensis* antigen. Interestingly, 89% of deer that were PCR negative for *E. chaffeensis* or *E. ewingii* also had positive titers in the *E. chaffeensis* antibody assay. This finding was presumably caused by cross-reacting antibodies, since reactivity with *E. chaffeensis* antigen has previously been observed for samples from deer infected with the WTD agent (7). Only two deer (3%) were positive for *A. phagocytophila* antibodies. Several deer were PCR positive but antibody negative. These animals may have been recently infected and had not yet mounted an antibody response or may have been chronically infected with no detectable antibody.

The role of white-tailed deer as a vertebrate reservoir of *E. chaffeensis* has been well documented by studies that included experimental infection, detection of natural infection, and experimental transmission among deer by the primary tick vector *Amblyomma americanum* (8, 11, 14). The presence of *E. chaffeensis*, *A. phagocytophila*, and the WTD agent in a single deer population indicates high levels of exposure to ehrlichiae within the wild deer herd (13) and demonstrates that individual deer can be simultaneously coinfected with all three agents.

E. ewingii has long been known to cause infections in dogs and was first observed in the blood of a dog in Arkansas (10) and later in Oklahoma (1, 10, 16). The natural history of *E. ewingii* is not as well understood as that of *A. phagocytophila* or *E. chaffeensis*. In experimental settings, *A. americanum* has been shown to be a competent vector (2), and natural infec-

TABLE 2. Correlation between PCR finding and presence of antibodies against *Ehrlichia* or *Anaplasma* species

Agents detected by PCR	No. of deer	No. (%) of deer with antibody against:	
		E. chaffeensis	*A. phagocytophila*
E. chaffeensis + *Anaplasma* spp.[a]	16	16 (100)	0
E. ewingii + *Anaplasma* spp.	18	18 (100)	0
E. chaffeensis + *E. ewingii* + *Anaplasma* spp.	13	13 (100)	0
Anaplasma spp. only	63	56 (89)	2 (3)
None	2	1 (50)	0
All deer	112	97 (87)	2 (2)

[a] Nucleotide sequences of select amplicons matched that of the WTD agent, an unclassified *Anaplasma*-like bacterium.

tions of white-tailed deer in Kentucky, Georgia, and South Carolina have been reported (20).

In the present study, virtually all of the deer (110 of 112 in 2000 and 104 of 105 in 2001) were actively infected with the WTD agent. Furthermore, 13 deer (12%) in 2000 and 8 (7%) in 2001 were infected with *E. chaffeensis*, *E. ewingii*, and the WTD agent at the time of their death. None of the 112 deer tested in the present study was positive for *E. canis*. This result was expected because previous studies have reported that *E. canis* does not establish infection or cause seroconversion in white-tailed deer (8).

On the basis of these results, we conclude that wild white-tailed deer in Missouri are important reservoirs of two ehrlichial agents that infect humans, *E. chaffeensis* and *E. ewingii*. We have shown that both *E. chaffeensis* and *E. ewingii* were detectable by PCR in 12 to 28% of deer killed during the hunting season in Missouri in 2000 and 2001. In addition, a large proportion of the deer were infected with the WTD agent, which has not been shown to cause human infection. The results of this study confirm a previous observation (13) that some PCR assays originally designed to detect *A. phagocytophila* 16S rDNA also detect the WTD agent. Further investigations into the animal reservoirs of ehrlichiae are in progress.

Nucleotide sequence accession numbers. The GenBank accession numbers for the WTD agent sequences used in comparisons are U27101, U27102, U27103, and U27104. These sequences contain several ambiguity codes. Therefore, exact homology cannot be determined for certain parts of the 16S rDNA sequence. The 16S rDNA sequence determined from deer blood samples in this study, and found to be similar to the previously deposited WTD agent sequences, was assigned accession number AY180920.

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