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Original article

Anaplasma odocoilei sp. nov. (family Anaplasmataceae) from white-tailed deer (*Odocoileus virginianus*)

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ABSTRACT

Recently, an undescribed *Anaplasma* sp. (also called *Ehrlichia*-like sp. or WTD agent) was isolated in ISE6 tick cells from captive white-tailed deer. The goal of the current study was to characterize this organism using a combination of experimental infection, morphologic, serologic, and molecular studies. Each of 6 experimentally inoculated white-tailed deer fawns (*Odocoileus virginianus*) became chronically infected (100+ days) with the *Anaplasma* sp. by inoculation of either infected whole blood or culture. None of the deer showed evidence of clinical disease, but 3 of the 6 deer evaluated had multiple episodes of transient thrombocytopenia. Light microscopy of Giemsa-stained, thin blood smears revealed tiny, dark, spherical structures in platelets of acutely infected deer. *Anaplasma* sp. was detected in platelets of inoculated deer by polymerase chain reaction, transmission electron microscopy, immunohistochemistry, and in situ hybridization. Five of 6 deer developed antibodies reactive to *Anaplasma* sp. antigen, as detected by indirect fluorescent antibody testing. Phylogenetic analyses of 16S rRNA, groESL, and gltA sequences confirmed the *Anaplasma* sp. is related to *A. platys*. Two attempts to transmit the *Anaplasma* sp. between deer by feeding *Amblyomma americanum*, a suspected tick vector, were unsuccessful. Based on its biologic, antigenic, and genetic characteristics, this organism is considered a novel species of *Anaplasma*, and the name *Anaplasma odocoilei* sp. nov. is proposed with UMUM76^T (=CSUR-A1) as the type strain.

Introduction

In the United States, white-tailed deer (WTD; *Odocoileus virginianus*) are infected with multiple tick-transmitted rickettsiae in the genera *Ehrlichia* and *Anaplasma* (Lockhart et al., 1997a; Little et al., 1998; Yabsley et al., 2002, 2008a). Three species, *E. chaffeensis*, *E. ewingii*, and *Anaplasma* (*A.*) *phagocytophilum* are zoonotic pathogens and infect a wide range of mammalian species (Dumler et al., 2001). A fourth organism, detected by PCR in the blood of wild WTD, has been referred to as WTD agent or *Ehrlichia* sp. of WTD (Dawson et al., 1996; Little et al., 1997) and was recently isolated in tick cell culture and identified as a member of the genus *Anaplasma* (Munderloh et al., 2003).

This *Anaplasma* sp. is common among white-tailed deer populations in the southeastern United States and has been reported from Georgia, Oklahoma, Nebraska, Virginia, and Missouri (Little et al., 1997; Lockhart et al., 1997b; Arens et al., 2003; Yabsley, unpublished). A closely-related species has been detected in mule deer (*O. hemionus hemionus*) and black-tailed deer (*O. hemionus columbianus*) from California (Foley et al., 1998; Yabsley et al., 2005) and mule deer and white-tailed deer from Arizona (Yabsley et al., 2005). However, additional data are needed to determine if this *Anaplasma* represents a variant of the *Anaplasma* sp. detected in white-tailed deer in the southeastern United States. In the southeastern United States, it has significant temporal and spatial associations with lone star tick (*Amblyomma americanum*) infestation (Brandsma et al., 1999). Additionally, this *Anaplasma* sp. has been detected by polymerase chain reaction (PCR) in field-collected *Amblyomma americanum* ticks from Missouri (GenBank

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Table 1
Oligonucleotide sequences of primers used in this study.

Primer pair	Gene target	Target organism(s)	Nucleotide sequence (5′→3′)	Product size (bp)	References
ECC ECB	16S rRNA	*Anaplasma* and *Ehrlichia* spp.	CGTATTACCGCGGCTGCTGGCA AGAACGAACGCTGGCGGCAAGCC	480	Dawson et al. (1994)
GE9F GA1UR	16S rRNA	*A. phagocytophilum* and *Anaplasma* sp. UMUM^T	AACGGATTATTCTTTATAGCTTGCT GAGTTTGCCGGGACTTCTTCT	411	Chen et al. (1994) and Little et al. (1997)
DGA GA1UR	16S rRNA	*Anaplasma* sp. UMUM^T	TTATCTCTGTAGCTTGCTACG GAGTTTGCCGGGACTTCTTCT	411	Little et al. (1997)
DGA T7-398R	16S rRNA	*Anaplasma* sp. UMUM^T	TTATCTCTGTAGCTTGCTACG T7-GCATAGCTGGATCAGGCTTTC	325	Dawson et al. (1994) and Little et al. (1997)
APF1 APR1	*groESL*	*A. phagocytophilum* and *Anaplasma* sp. UMUM^T	TAGTGATGAAGGAGAGTGAC CCAGGIGCCTTIACAGCWGCAAC	1603	Sumner et al. (2003)
EDF10 EDR11	*groESL*	*Anaplasma* sp. UMUM^T	GATTCTCCGGTTTGTTCTGT GGAGAAAGATAACCCCTG	650	Sumner et al. (2003)
F4b HG1085R	*gltA*	*Anaplasma* and *Ehrlichia* spp.	CCGGGTTTTATGTCTACTGC ACTATACCKGAGTAAAAGTC	935	Inokuma et al. (2001, 2002)
16S+1 16S−1	16S rRNA	Tick	CCGGTCTGAACTCAGATCAAGT CTGCTCAATGATTTTTTAAATTGCTGTGG	460	Norris et al. (1999)

accession number ELU52514). These previous studies suggest that this *Anaplasma* sp. infects white-tailed deer and could be transmitted by *Amblyomma americanum*, similar to 2 important zoonotic rickettsiae, *E. chaffeensis* and *E. ewingii* (Ewing et al., 1995; Little et al., 1998; Yabsley et al., 2003). The significance of this organism as a pathogen of deer or as a potential zoonotic agent is currently unknown. Previous analysis of partial 16S rRNA and GroESL gene sequences of this *Anaplasma* sp. indicates that it is most closely related to *A. platys*, a canine rickettsia that infects platelets of dogs and potentially humans (Dawson et al., 1996; Arraga-Alvarado et al., 1999; Dumler et al., 2001; Sumner et al., 2003).

In the current study, we experimentally infected deer with this *Anaplasma* sp. in order to (i) investigate infection dynamics and cellular tropism of this organism in vivo, (ii) conduct a small-scale *Amblyomma americanum* transmission study, and (iii) more fully characterize the morphologic, molecular, and antigenic relationships of this so far undescribed *Anaplasma* sp.

Materials and methods

Experimental animals and procedures

Nine orphaned white-tailed deer fawns (WTD76, 77, 81, 86, 128, and 135, and Deer 1, 2, and 3) were hand-raised and housed in a tick-free building at the College of Veterinary Medicine, UGA, Athens, GA. Fawns were acquired within 1–3 days of birth, and prior to inoculation, tested negative for *E. chaffeensis*, *E. ewingii*, *A. phagocytophilum*, and the *Anaplasma* sp. by PCR and negative for antibodies to *E. chaffeensis* and *A. phagocytophilum* by indirect fluorescent antibody (IFA) assays as described (Dawson et al., 1994, 1996; Lockhart et al., 1997a; Little et al., 1998; Yabsley et al., 2002; Munderloh et al., 2003). For inoculations, deer were anesthetized by intramuscular injection of 1.7 mg/kg of xylazine (Mobay Corporation, Shawnee, KS) and 0.1 mg/kg ketamine (Fort Dodge Laboratories Inc., Fort Dodge, IA) and reversed with 1.3 mg/kg of yohimbine (Lloyd Laboratory, Shenandoah, IA).

Following inoculation, whole blood was collected periodically for PCR, serologic tests, and blood smears. Complete physical examination of each deer was performed at each blood collection date (Blood et al., 1983). Periodically, whole-blood anticoagulated in ethylenediaminetetraacetic acid (EDTA) from WTD76, WTD81, and WTD77 was submitted to the Clinical Pathology Laboratory, College of Veterinary Medicine, The University of Georgia, for analysis of the following parameters: hematocrit, erythrocyte count, hemoglobin levels, platelet count, total and differential leukocyte counts, and fibrinogen. At the end of the study, all deer were anesthetized as described above, euthanized via intravenous sodium pentobarbital overdose, and a necropsy performed. All procedures were approved by the UGA Institutional Animal Care and Use Committee (A2004-10136).

To detect *Anaplasma* sp. in blood of experimental deer and ticks, RNA was extracted from whole blood with the RNA Blood Minikit (Qiagen, Inc., Valencia, CA) and ticks with the QiAmp® Viral RNA Extraction Kit (Qiagen). For ticks, individuals were frozen in liquid nitrogen and then macerated with glass beads in a Mini Beadbeater-8 (Biospec Products, Inc., Bartlesville, OK). Reverse transcriptase (RT)-nested PCR (nPCR) assays for the 16S rRNA and groESL genes were performed as described (Munderloh et al., 2003; Tate et al., 2005); except that for the groESL assay, secondary amplification was performed using primers EDF10 and EDR11 (Sumner et al., 2003) (Table 1). As a control for the quality of the RNA preparations from frozen ticks, a random subset were tested for the presence of tick RNA using primers 16S+1 and 16S−1 (Table 1) which amplify a fragment of the tick mitochondrial 16S rRNA (Norris et al., 1999). Amplification products were separated by electrophoresis in a 2% agarose gel stained in ethidium bromide and visualized using ultraviolet transillumination.

Experimental inoculation trials

Details of fawn numbers and source of infections are summarized in Table 3. Briefly, two 4-month-old fawns (WTD76 and WTD81) became infected with the *Anaplasma* sp. after inoculation with blood collected from field-collected deer as described (Yabsley et al., 2002; Munderloh et al., 2003). Whole blood (8.0 ml) in EDTA collected from WTD 76, the original source of UMUM76^T, at 177 DPI was used to inoculate one 10-month-old deer (WTD 77) by each of 4 routes (2 ml each): intradermal, subcutaneous, intravenous, and intraperitoneal (ID, SQ, IV, IP). A 13-month-old deer (WTD 86)

Table 2
GenBank accession numbers used in phylogenetic analyses.

Organism	16S rRNA	*groESL*	*gltA*
Anaplasma sp. UMUM^T	JX876644	JX876642	DQ020101
Anaplasma platys	AF536828	AY044161	AB058782
Anaplasma phagocytophilum	AY055469	AF172161	AF304136
Anaplasma marginale	AF309867	AF414865	AF304140
Ehrlichia ruminantium	X62432	U13638	AF304146
Ehrlichia ewingii	EEU96436	AF195273	DQ365879
Ehrlichia chaffeensis	AF147752	L10917	AF304142
Ehrlichia canis	AF373613	U96731	AF304143
Neorickettsia sennetsu	M73225	ESU88092	AF304148