AN ASSESSMENT OF THE ROLE OF WHITE-TAILED DEER IN THE
EPIZOOTOLOGY OF ANAPLASMOSIS IN THE
SOUTHEASTERN UNITED STATES

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ABSTRACT: The role of white-tailed deer (Odocoileus virginianus) in the epizootiology of anaplasmosis in the southeastern United States was examined through retrospective and prospective serosurveys and by experimental infection studies. No serum antibody reactive to Anaplasma marginale was detected with an indirect fluorescent antibody (IFA) assay from any of 1,376 free-ranging deer sampled from 1968 through 1990 from 13 states and Puerto Rico. Thirty-one additional deer from three bovine anaplasmosis enzootic premises also were negative by IFA and Giemsa-stained blood films. Three captive deer given A. marginale intravenously developed antibodies 38 to 41 days post-inoculation (DPI) and remained seropositive for the duration of the study (161 to 287 DPI). At 42 DPI, rickettsias of approximately 0.001% infected erythrocytes were observed in all three deer using a DNA probe; low rickettsias (maximum 0.01%) persisted through 56, 63, and 87 DPI, respectively. One deer had a recrudescent infection from 126 to 146 DPI (maximum rickettsemia 0.001%). We believe that white-tailed deer in the southeastern United States, even though susceptible to A. marginale infection, are not exposed naturally, even at enzootic sites. Furthermore, white-tailed deer did not develop rickettsemias sufficient to support mechanical transmission by biting flies, which is believed to be the primary means of anaplasmosis transmission in this region.

Key words: Anaplasmosis, Anaplasma marginale, white-tailed deer, Odocoileus virginianus, serology, survey, experimental infection.

INTRODUCTION

Bovine anaplasmosis is a vector-borne disease caused by the obligate intraerythrocytic rickettsia, Anaplasma marginale. Lost productivity and mortality due to anaplasmosis have had serious financial consequences in the cattle industry (Alderink and Dietrich, 1983). Financial costs are attributed to material losses of cattle and productivity as well as the cost of treatment and vaccination.

Although A. marginale is primarily a pathogen of cattle (Losos, 1986), it can infect a wide variety of ruminants. In animals other than cattle, A. marginale typically produces only a mild infection that is clinically inapparent. In North America, A. marginale occurs naturally in mule deer (Odocoileus hemionus) and black-tailed deer (O. h. columbianus) (Boynton and Woods, 1953; Christensen et al., 1960; Howe and Hepworth, 1965). American bison (Bison bison) are experimentally susceptible to A. marginale and develop clinical disease similar to that in cattle, but natural infections have not been reported (Zaugg, 1986). Pronghorn antelope (Antilocapra americana), bighorn sheep (Ovis canadensis), and elk (Cervus elaphus) also are experimentally susceptible, but naturally occurring infections have not been confirmed in any of these species (Howe et al., 1964; Renshaw et al., 1979).

In the United States, anaplasmosis is enzootic among cattle in the intermountain West, California, and the Southeast (McCallon, 1973). The epizootiology of the disease is well documented in the two western enzootic areas where A. marginale is biologically transmitted by ticks, particularly by species of the genus Dermacentor, and A. marginale is known to naturally infect both mule deer and black-tailed deer. Neither species of deer devel-
ops clinical illness, but black-tailed deer seem to maintain higher rickettsias and have been implicated as a reservoir host (Osbold et al., 1959; Howarth et al., 1976).

In the southeastern U.S. the epizootiology of A. marginale is much less certain. This region appears to lack suitable species of tick vectors, and mechanical transmission is believed to occur by fomites or biting flies. The inefficiency of mechanical transmission necessitates the close proximity of infected and susceptible hosts if successful inoculation is to occur (Howell et al., 1941).

The possible involvement of white-tailed deer in the maintenance and spread of anaplasmosis in the eastern United States always has been unclear, partly because of the unreliability of traditional serologic tests when applied to deer serum (Kuttler, 1981). Based on their subinoculation studies, Bedell and Miller (1966) reported that white-tailed deer from southeastern states were not involved in the epizootiology of this disease. However, since that time, white-tailed deer populations have increased dramatically in both density and distribution, and the chance of contact between deer and infected cattle has increased proportionately. These changes, coupled with knowledge of the role of deer in the epizootiology of anaplasmosis in the western United States, have served to perpetuate concerns that white-tailed deer may be a source of infection for cattle.

MATERIALS AND METHODS

Serum samples for a retrospective serological survey were obtained from the serum bank of the Southeastern Cooperative Wildlife Disease Study, Department of Parasitology, College of Veterinary Medicine, The University of Georgia, Athens, Georgia (USA). These samples were from 1,376 white-tailed deer collected in Alabama (n = 156), Arkansas (n = 143), Florida (n = 259), Georgia (n = 346), Kentucky (n = 15), Louisiana (n = 171), Maryland (n = 10), Mississippi (n = 32), North Carolina (n = 31), Puerto Rico (n = 5), South Carolina (n = 33), Tennessee (n = 53), Virginia (n = 35), and West Virginia (n = 87). The region in the U.S. from which these samples were collected ranged from 19°53' to 40°39'N, and from 74°9' to 94°37'W. Five deer were collected in 1968 and the remaining 1,371 were collected from 1980 through 1990. Sample size per location ranged from one to 47 (mean ± SD = 6.9 ± 5.5), and deer were sampled at 145 locations representing 137 counties.

The maximum potential prevalence of seropositive deer was estimated using the upper limit of a 95% confidence interval constructed around the number of individuals in the sample that were seropositive, assuming a binomial distribution (Steel and Torrie, 1980). This estimation procedure assumes a 100% sensitivity of the serologic testing procedure.

Enzootic areas of bovine anaplasmosis were located through contact with diagnostic laboratories, regulatory veterinarians, and state wildlife agency personnel. In reviewing premises identified as having bovine anaplasmosis, care was taken to ensure that the clinical history of the cattle indicated a truly enzootic site. At each location, clinical anaplasmosis had been diagnosed for at least two consecutive years.

In 1990 and 1991, white-tailed deer were collected by shooting at three such premises: Nelson County, Kentucky (37°46'N, 85°29'W), 1991 (n = 9); Granville County, North Carolina (36°24'N, 78°59'W), 1991 (n = 8); and Marion County, Tennessee (35°3'N, 85°40'W), 1990 (n = 5) and 1991 (n = 9). Blood samples obtained by cardiac puncture were collected in ethylenediaminetetraacetic acid (EDTA) for the preparation of thin blood films and for testing by DNA hybridization (1990 only). Whole blood was also collected, allowed to clot, and the serum used for serologic testing.

Five white-tailed deer fawns, seronegative for A. marginale, were obtained from The University of Georgia’s Daniel B. Warnell School of Forest Resources’ captive herd at Whitehall Experimental Forest, Athens, Georgia. Three fawns approximately 8 mo old were inoculated intravenously with a stablate of a Virginia isolate of A. marginale (S_m-V, Am) provided by the Animal Disease Research Unit, Agriculture Research Service, U.S. Department of Agriculture, Pullman, Washington (USA). The two remaining deer served as uninfected controls. The deer were anesthetized with a mixture of 1.7 mg/kg ketamine (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, USA) and 0.1 mg/kg xylazine (Mobay Corporation, Shawnee, Kansas, USA) administered intramuscularly, and catheters (Delmed, Inc., Canton, Massachusetts, USA) were inserted into the jugular veins for efficient administration of the stablate. The stablate was thawed and warmed in the hand, diluted 1:10 with normal deer serum, and immediately administered via the catheter. Administering 4 ml of the stablate:serum solution resulted in an
inoculum of $5 \times 10^8$ infected erythrocytes. A splenectomized calf, which served as a control to test the viability of the rickettsial stablate, was simultaneously given an identical dose of the stablate with the exception that normal bovine serum was used as a diluent. The control deer were inoculated with an identical volume of sterile saline: deer serum solution.

Once inoculated, all animals were housed inside in a manner that prevented contact with potential arthropod vectors. Uninfected control deer were housed in the same rooms with the infected deer. Blood samples were obtained from all experimental animals periodically from 0 to 146 days post-inoculation (DPI) to obtain serum, make thin blood films, monitor packed cell volumes (PCV), and collect washed erythrocytes for DNA hybridization. Control deer were maintained for 146 and 154 DPI. Infected deer were maintained 161 to 287 DPI. As soon as the calf developed a rapidly increasing, microscopically detectable rickettsemia concurrent with hemolytic anemia, it was euthanized with a Supercash Mark 2 penetrating bolt (Aecles & Sherlock Ltd., Aston-Birmingham, England).

An indirect fluorescent antibody (IFA) assay was used to screen deer sera for antibodies to *Anaplasma* spp. We used a fluorescein-labeled protein-G to detect serum IgG specific for *A. ovis* or *A. marginale*, including all isolates of either species (Tibbitts et al., 1992). Antigen slides for the assay were uniform thin blood smears of bovine erythrocytes infected with the S$_m$-V$_r$, Am strain of *A. marginale*. All antigen slides were prepared simultaneously from a single blood sample, air dried, wrapped in aluminum foil, and immediately stored frozen at $-20^\circ$C until used. All serum samples were centrifuged (15,000 $\times$ G) for 10 min to remove suspended lipids or any precipitates, then diluted 1:100 in phosphate buffered saline solution prior to testing. Positive control serum from an experimentally infected calf and negative control serum from a captive white-tailed deer were included in each IFA assay. The IFA slides were examined using an Olympus BH-2 microscope (Olympus Optics Co., Ltd., Tokyo, Japan) with a 40 $\times$ oil immersion objective with a 1:1 mixture of glycerin : distilled water as an immersion fluid. Illumination was with a Mercury-100 super pressure mercury vapor lamp (Chiu Technical Corporation, Kings Park, New York, USA).

Routine thin blood films from the prospective and experimental infection studies were fixed for 3 min in 100% methanol, stained for 20 to 30 min in a 1:25 dilution of Giemsa stain in distilled water, rinsed with distilled water, and air-dried. Stained slides were examined microscopically for *A. marginale* using a 100 $\times$ oil immersion objective.

Rickettsemias in experimental deer were measured directly using a DNA probe as described by Goff et al. (1990). Rickettsemias were determined by comparing the probe response for experimental samples with the probe response to serial dilutions of a positive control with a known rickettsemia. The probe is specific for *A. marginale* and can detect a rickettsemia as low as 0.000025% infected erythrocytes. The sensitivity and specificity of the probe enabled assessment of the concentrations of the rickettsemia below levels that were detectable by the microscopic analysis of stained blood films. The DNA probe also was used with blood samples from five deer collected from Marion County, Tennessee during 1990 for the prospective survey. Binomial confidence limits were determined by the methods of Steel and Torrie (1980).

**RESULTS**

No specific serum antibody was detected among any of the 1,376 white-tailed deer from 13 states and Puerto Rico in the retrospective survey. Based on a 95% confidence interval with a binomial distribution, the maximum possible prevalence of seroreactors with 1,376 consecutive negative results would be 0.27% for the combined regional deer population. Maximum possible prevalence of seroreactors using data for individual states where $\geq$30 deer were sampled ranged from 1 to 11%.

All of the 31 deer sampled in the prospective survey from three anaplasmosis enzootic premises also were seronegative, and *A. marginale* was not detected in Giemsa-stained blood films. In addition, all five deer sampled from the Marion County, Tennessee, premise in 1990 were DNA-probe negative.

The *A. marginale* stablate used for experimental inoculations was fully infective. Intraerythrocytic *A. marginale* inclusion bodies first were detected in the splenectomized calf at 31 DPI. At that time, the PCV was stable at 34%. By 35 DPI, a 31% rickettsemia had developed with multiple inclusion bodies in most infected erythrocytes, and the PCV had decreased to 21%.

The three inoculated deer developed *A. marginale*-specific circulating antibodies.
detectable with the IFA assay at 38 to 41 DPI. All three animals remained seropositive for the duration of the study (161 to 287 DPI) (Table 1). The two control deer remained seronegative through 146 and 154 DPI, at which time they were removed from the study.

On microscopic examination of Giemsa-stained blood films we did not find *A. marginale* in any of the experimentally inoculated deer. Using the DNA probe, however, we discovered low-level rickettsemias in each of the three inoculated deer (Table 1) commencing at 42 DPI (Fig. 1). Two deer had single episodes of rickettsemia from 42 to 87 DPI and 42 to 63 DPI with maximum rickettsemias of 0.01% and 0.001%, respectively (Fig. 1). Thereafter, these two deer were consistently negative on multiple DNA probe tests conducted through 161 DPI and 287 DPI, respectively. Similar to the other two infected deer, the third deer resolved the initial rickettsemia at 56 DPI; however, this deer experienced a recrudescent rickettsemia from 126 to 146 DPI (Fig. 1). Eight sub-

### Table 1.

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<th>Animal number</th>
<th>Status</th>
<th>First time seropositive</th>
<th>Last time seropositive</th>
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* Days postinoculation.

* As determined by DNA probe; all negative by light microscopy of Giemsa-stained blood films.

![Figure 1](http://example.com/figure1.png)

**Figure 1.** Rickettsemias in white-tailed deer experimentally infected with *Anaplasma marginale* as indicated by a DNA probe. All deer were consistently negative from 19-41 weeks postinfection.
sequent samples obtained from this animal between 154 and 287 DPI were uniformly negative when examined with the DNA probe. The maximum rickettsemia detected in the third deer was 0.001% during both the initial and recrudescent rickettsemias. Experimentally infected deer did not develop anemia or other clinical signs of anaplasmosis.

**DISCUSSION**

A lack of sensitivity and specificity in diagnostic assays has compromised most past serologic surveys of white-tailed deer for exposure to *A. marginale* (Kuttler, 1981). The IFA assay used in this survey was validated using experimentally infected deer as well as uninoculated controls. The accurate identification of experimentally infected, spleen-intact white-tailed deer by the IFA assay and the length of time which these animals remained seropositive are good evidence for the applicability of the IFA assay in surveying wild white-tailed deer. Tibbitts et al. (1992) and Jessup et al. (1993) demonstrated the usefulness of the IFA assay for *Anaplasma* serology with other species of wild ruminants.

Both the retrospective region-wide serosurvey and the prospective survey of enzootic premises failed to show naturally occurring *A. marginale* infections among white-tailed deer in the southeastern United States. These results are in agreement with subinoculation studies on white-tailed deer conducted 28 yr earlier (Bedell and Miller, 1966) when deer populations were much lower than they are currently. Based on these findings, we propose that the expanded geographic distributions and increased densities of most deer populations have not resulted in a change in the status of white-tailed deer in the epizootiology of *A. marginale* in this region. Using serosurveys, others also have shown that white-tailed deer are unimportant in the epizootiology of *A. marginale* (Maas et al., 1981; Morley and Hugh-Jones, 1989), but most of these surveys were conducted with diagnostic tests of limited reliability in deer (Kuttler, 1981).

The experimentally inoculated white-tailed deer had evidence for an inherent ability to control *A. marginale* infections and developed inappreciable rickettsemias with no indication of clinical disease. These findings are in agreement with the few previous experimental *A. marginale* infections of spleen-intact white-tailed deer (Kuttler, 1981). The recrudescent rickettsemia in one deer is evidence of a potential for low levels of cyclic rickettsemia, as occurs in some carrier cattle (Kieser et al., 1990); however, the rickettsemias in all deer were much lower than those reported for spleen-intact, infected cattle. Kuttler et al. (1967) demonstrated latent persistence of *A. marginale* in an experimentally infected white-tailed deer which developed a relapsing infection following splenectomy at 179 DPI. Collectively, these studies are evidence that, like mule deer and black-tailed deer, white-tailed deer have the biologic capability to become inapparent carriers of *A. marginale*.

Based on experimental infection studies, white-tailed deer are biologically capable of serving as reservoir hosts for *A. marginale*; yet there is no evidence that they function in this capacity in the southeastern United States, based on surveys of free-ranging white-tailed deer. This discrepancy between potential and actual reservoir status focuses attention on the vectors of anaplasmosis in this region. The primary vectors of bovine anaplasmosis in the southeastern United States are believed to be biting flies, especially members of the family Tabanidae (Piercy, 1956; Wilson and Meyer, 1966), which are mechanical rather than biological vectors (Kuttler, 1981).

Mechanical transmission by biting flies is considered much less efficient than biological transmission by ticks (Kuttler, 1981). Numerous constraints reduce the efficiency of mechanical transmission by biting flies: microscopically detectable rickettsemias are necessary for tabanid
transmission of A. marginale between cattle (Lotze, 1944; Wilson and Meyer, 1966); multiple fly bites (≥10 bites) on recipient cattle are required for transmission, regardless of the level of rickettsemia in donor cattle (Howell et al., 1941; Lotze, 1944; Hawkins et al., 1981). A. marginale remains viable on the mouthparts of flies only briefly (≤5 min) (Howell et al., 1941; Lotze and Yiengst, 1941); only a very small amount of residual blood (10^{-6} to 10^{-5} ml) can be deposited by tabanids in a subsequent feeding (Foil et al., 1987); tabanids feed on pooled blood exiting bite sites (Foil et al., 1987) so that bleeding of bite sites may further reduce amount of infected blood that actually contaminates the host's circulation; and when tabanids are dislodged during feeding they tend to complete their bloodmeal on the same cow, even if other cattle are immediately adjacent (Sheppard, 1975). In addition, most use of cattle pastures by deer is at night when tabanids are inactive. For these reasons, mechanical transmission by tabanids from cattle to deer is highly improbable. These factors also would act to preclude transmission among deer.

The current absence of known tick vectors in the southeastern United States appears to be the critical difference between the role of white-tailed deer in the epizootiology of anaplasmosis in this region and that of both black-tailed deer and mule deer in the western United States. Four species of ticks (Amblyomma americanum, A. maculatum, D. albipictus, Ixodes scapularis) are commonly found on white-tailed deer in the Southeast (Kellogg et al., 1971; Smith, 1977). Amblyomma americanum, A. maculatum and I. scapularis were not capable of transmitting A. marginale in experimental infection trials, thus eliminating these ticks as potential vectors (Rees, 1934; Sanborne and Moe, 1934; Anthony and Roby, 1966; Lancaster et al., 1968). Only D. albipictus was able to transmit the rickettsia when experimentally transferred from infected to susceptible hosts (Stiller et al., 1981). However, because it is a one-host tick, D. albipictus is unlikely to infest multiple hosts under natural conditions.

In the absence of biological tick vectors, meaningful involvement of white-tailed deer in the epizootiology of A. marginale in the southeastern United States is very unlikely. However, the introduction of an efficient biological tick vector could alter both the occurrence of anaplasmosis among cattle and the role of white-tailed deer in the epizootiology of A. marginale. Therefore, prevention of the introduction and establishment of suitable tick vectors in this region is of critical importance.

ACKNOWLEDGMENTS

Major funding for this study was provided by The University of Georgia’s Veterinary Medical Experiment Station. Supplemental support was through the Federal Aid in Wildlife Restoration Act (50 Stat. 917), through Contract Numbers 14-16-0004-91-913 and 14-16-0004-92-909, Fish and Wildlife Service, U.S. Department of the Interior, and through Contract Number CWWU-5348-32000-008-00D, Agricultural Research Service, U.S. Department of Agriculture. A graduate research assistantship (Keel) was provided by the Daniel B. Warnell School of Forest Resources. Sponsorship by the fish and wildlife agencies of Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, Missouri, North Carolina, Puerto Rico, South Carolina, Tennessee, Virginia, and West Virginia also made this study possible. The authors thank B. Cowles, T. E. Creekmore, W. C. Johnson, K. A. Lee, and L. M. White for technical assistance. The Tennessee Wildlife Resources Agency, the Kentucky Department of Fish and Wildlife Resources; and the North Carolina Wildlife Resources Commission assisted with deer collections for the prospective study.

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Received for publication 15 March 1994.