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SEROEPIDEMIOLOGY OF *ANAPLASMA MARGINALE* IN WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) FROM LOUISIANA

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ABSTRACT: Antibodies to *Anaplasma marginale* were detected by the indirect fluorescent antibody test (IFA) in six of 331 (2%) serum samples of white-tailed deer (*Odocoileus virginianus*) from Louisiana. None of the serum samples were positive using the *A. marginale* modified rapid card agglutination test. Of the six IFA positive sera retested by the complement fixation test four sera gave anticomplementary and two gave seropositive reactions. The low *A. marginale* reactor rate in this white-tailed deer population was probably a reflection of the lack of cohabitation between cattle and deer and the fact that the primary arthropod vectors in Louisiana are tabanids. The validity of the indirect fluorescent antibody test for *A. marginale* antibodies in white-tailed deer should be evaluated.

Key words: White-tailed deer, *Odocoileus virginianus*, *Anaplasma marginale*, serosurvey, indirect fluorescent antibody test, modified rapid card agglutination test.

INTRODUCTION

Seroepidemiology of anaplasmosis in white-tailed deer (*Odocoilus virginianus*) has been conducted in areas where anaplasmosis is enzootic in cattle. Bedell and Miller (1966) failed to demonstrate the presence of *Anaplasma marginale* infection of 262 adult white-tailed deer from seven southeastern states in the United States by subinoculation of deer blood into susceptible calves. Of 30 serum samples collected from white-tailed deer in three counties in Texas (USA), 14 (47%) were seropositive to the capillary tube agglutination (CTA) test (Robinson et al., 1968). In Missouri (USA), seven (1%) of 616 sera obtained from hunter-killed white-tailed deer were positive to the *A. marginale* modified rapid card agglutination (MRCA) test (Maas and Buening, 1981). Sera collected from 175 live-trapped and hunter-killed white-tailed deer in and adjacent to a wildlife refuge in southern Illinois (USA) revealed 12 (7%) seropositive to the CTA test. Of 54 serum samples obtained from cattle that grazed within the refuge seven (13%) were seropositive (Smith et al., 1982).

Study of the deer population in Louisiana (USA) for *A. marginale* infection has been limited to a component of the one conducted by Bedell and Miller (1966). The 1984 estimate of the white-tailed deer population in Louisiana was between 500,000 to 750,000 (Game Division, Louisiana Department of Wildlife and Fisheries, Baton Rouge, Louisiana 70803, USA). This large deer population could provide *A. marginale* carrier animals to influence the epidemiology of bovine anaplasmosis. The purpose of this study was to determine the seroprevalence of *A. marginale* antibodies in white-tailed deer in Louisiana. A secondary objective was to compare two serological tests, the indirect fluorescent antibody (IFA) test and the MRCA test, for the detection of *A. marginale* antibodies in white-tailed deer serum.

MATERIALS AND METHODS

Deer serum samples

Convenience sampling was employed. A serum bank of white-tailed deer sera frozen at −18 C since collection during 1980 to 1984 was used. The sera had been collected as part of an ongoing annual survey conducted by the Department of Veterinary Pathology (School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana 70803, USA) and sponsored by the Louisiana Department of Wildlife and Fisheries. The deer had been hunt-

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er-killed at 38 sites, mainly wildlife management and hunt club areas, within 27 of the 64 parishes of Louisiana. The deer had been aged on the basis of tooth eruption and wear, weight, size and antler growth in male deer. Of 331 sera made available for this study 77 had been obtained from deer collected during 1980, 100 during 1981, 89 during 1982, 24 during 1983, and 41 collected during 1984. The sex distribution of the deer in this sample was 185 female, 103 male, and 43 unspecified. The age distribution of the deer was 13 <1 yr, 135 at 1 to 1.5 yr, 86 at 2 to 2.5 yr, 28 at 3 to 3.5 yr, 19 ≥4 yr, and 50 unspecified.

After allowing the sera to thaw at room temperature for 18 hr, each serum vial was inverted to mix the sample before testing for *A. marginale* antibodies by the IFA test. The serum samples were held for an additional 6 hr at room temperature before performing the MRCA test.

Indirect fluorescent antibody test

The IFA antigen was prepared from *A. marginale* infected erythrocytes according to the methods of Gonzalez et al. (1978) and Goff and Winward (1983), with modifications. Rabbit anti-bovine IgG fluorescein conjugated whole molecule (Miles Laboratories, Inc., Naperville, Illinois 60566, USA) was used at a dilution of 1:80 with phosphate buffered saline (PBS).

Anoplasma marginale was passaged serially through two splenectomized calves. Moderately thick blood smears were made from PBS washed erythrocytes at a packed cell volume of 20% and stored at −40 C. Eighteen test sera diluted 1:10 with PBS plus a positive and a negative control deer serum (supplied by R. Smith, Department of Pathobiology, School of Veterinary Medicine, University of Illinois, Urbana-Champaign, Illinois 61801, USA) were tested on each blood-antigen slide. The slides were incubated at 37 C in a humid atmosphere for 45 min. After incubation the slides were rinsed with cold PBS and then washed in PBS for 15 min. The slides were drained and allowed to dry at room temperature. Approximately 15 μl of diluted conjugate was placed in each well and incubated in a second moist chamber at 37 C for 45 min. Finally, the slides were washed as after the first incubation and placed in cold PBS until read as positive or negative under a fluorescent microscope. Positive samples were retested at serum dilutions from 1:20 to 1:1,280.

Modified rapid card agglutination test

The MRCA tests were conducted according to the directions of the commercially available test kit (Anaplasmosis Card Test, Brewer Diagnostic Kit, Becton-Dickinson, Hunt Valley, Maryland 21030, USA). Characteristic clumping was read as positive for *A. marginale* antibodies. A positive and a negative control deer serum were used to test the reactivity of the MRCA test.

TABLE 1. Complement fixation (CF) and modified rapid card agglutination (MRCA) test results of six deer sera positive by the indirect fluorescent antibody (IFA) test for *Anaplasma marginale* antibodies.

Deer number (yr)	IFA titer	CF titer	MRCA result
512 (1980)	1:80	AC[a]	−[b]
519 (1980)	1:160	AC	−
656 (1981)	1:320	AC	−
683 (1981)	1:640	1:20	−
736 (1982)	1:20	AC	−
745 (1982)	1:20	1:5	−

[a] AC, anticomplementary.
[b] −, negative.

Complement fixation test

Deer serum samples found positive with the IFA test were complement fixation (CF) tested for *A. marginale* antibodies at the Louisiana Department of Agriculture Veterinary Diagnostic Laboratory, Louisiana State University (U.S. Department of Agriculture, 1973).

RESULTS

Six serum samples reacted positively to the IFA test while none reacted to the MRCA test. The observed agreement between the two tests was 98%. Four of the six IFA positive sera presented anticomplementary reactions to the CF test whereas the other two gave 1:5 and 1:20 titers (Table 1). The distribution of the six IFA positive sera as to year of collection was two in 1980, 1981 and 1982. The sera were taken from two male, two female, and two deer of unspecified sex. Two deer were collected from the same parish and the remaining four deer were from different parishes.

DISCUSSION

The 2% reactor rate to the IFA test is an estimate of the prevalence of *A. marginale* infection in white-tailed deer in Louisiana. This estimate is considerably less

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than the prevalence of 5% in the cattle population in Louisiana (Hugh-Jones et al., 1988). The fact that the deer had been hunter-killed in wildlife management and hunt club areas probably is reflected in the low reactor rate. How much cohabitation exists between cattle and deer in Louisiana and hence how much influence infection in white-tailed deer has on the epidemiology of bovine anaplasmosis, is not known.

Tabanidae are the primary vectors of bovine anaplasmosis in Louisiana (Wilson et al., 1963, 1970). Maas and Buening (1981) considered that the low prevalence of *A. marginale* antibodies in white-tailed deer of Missouri was partly because less efficient vectors, the horseflies, were the primary vector rather than ticks. Transmission of anaplasmosis from acutely infected calves to susceptible splenectomized calves has been accomplished with as few as 10 horsefly bites. These flies were shown to remain mechanically infective for at least 2 hr after they had obtained a partial blood meal from an acutely infected calf (Hawkins et al., 1981).

When Wilson and Richardson (1969) looked at the blood meals of tabanids collected in two areas of Louisiana, while there were no cattle-deer mixed blood meals, there were cattle-horse blood meals found. The tabanids are capable of flying considerable distances; flight ranges of marked *Tabanus lineola* and *Tabanus fusciostatus* are up to 6.8 km (Sheppard and Wilson, 1976). It is questionable, however, whether a partially fed tabanid persistent in completing its blood meal would fly from a cattle pasture to a deer habitat. Rather, the persistent tabanid would probably return to feed on the same host or a nearby host. In Louisiana, bovine anaplasmosis is initially a disease within herds, not an area disease (Hugh-Jones et al., 1988).

Serologic tests developed for anaplasmosis in cattle have been investigated for their validity in the detection of *A. marginale* antibodies in white-tailed deer and other game animals in North America. Using the CF test on 30 white-tailed deer sera Howe and Hepworth (1965) observed 25 anticomplementary and two false-positive reactions. A comparison of the CF and CTA tests on sera from known infected and uninfected white-tailed deer revealed sensitivity and specificity estimates of 94 and 100% for the CTA test and 100 and 54% for the CF test, respectively. A few anticomplementary reactions were observed with the CF test. The false-positive reactions of the CF test were mostly of lower titer (Kuttler et al., 1968; Kuttler, 1981).

Problems of low sensitivity or low specificity of the CF or CTA test have been observed upon testing sera of Columbian black-tailed deer (*Odocoileus hemionus columbianus*), mule deer (*Odocoileus hemionus hemionus*), pronghorn antelope (*Antilocapra americana americana*), elk (*Cervus elaphus nelsoni*), and bighorn sheep (*Ovis canadensis canadensis*) (Christensen et al., 1959; Post and Thomas, 1961; Howe et al., 1964; Howe and Hepworth, 1965; Magonigle et al., 1975; Howarth et al., 1976; Jacobson et al., 1977; Renshaw et al., 1977; Kuttler, 1984). The MRCA test correctly identified the sera of 21 known infected and 14 non-infected Columbian black-tailed deer, exhibiting this test's potential as a serologic test of high validity for this species (Howarth et al., 1976).

Use of the IFA test has been reported on testing sera of game animals in Africa (Lohr and Meyer, 1973; Lohr et al., 1974). Its validity was not determined. Gonzalez et al. (1978) had compared the IFA, CF, and MRCA tests for the diagnosis of bovine anaplasmosis and found that the IFA test was the most sensitive (97%). The CF and MRCA tests had sensitivities of 79 and 84%, respectively. The specificities of the IFA, CF, and MRCA tests were 90, 100, and 98%, respectively. Although the IFA test was employed in this study because of its reported high sensitivity in the detection of bovine anaplasmosis, the sensitivity and specificity of this test on the sera of white-

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tailed deer and other game animals must be evaluated. The reported higher sensitivity of the IFA test as compared to the MRCA test was observed in the results of this study.

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