SEROLOGICAL PREVALENCE AND ISOLATION OF BABESIA ODOCOILEI AMONG WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) IN TEXAS AND OKLAHOMA

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SEROLOGICAL PREVALENCE AND ISOLATION OF *BABESIA ODOCOILEI* AMONG WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) IN TEXAS AND OKLAHOMA

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ABSTRACT: Serum samples collected from 581 white-tailed deer (*Odocoileus virginianus*) from Texas and from 124 white-tailed deer from Oklahoma were tested by the indirect fluorescent antibody technique against *Babesia odocoilei*. Prevalence of seropositive reactors varied from site to site in both states. Prevalence rates were statistically ranked as high, intermediate or low. Deer <12-mo-old had a significantly lower prevalence than all other age classes.

Key words: White-tailed deer, *Odocoileus virginianus*, babesiosis, *Babesia odocoilei*, serological survey, prevalence.

INTRODUCTION

Spindler et al. (1958) described the first case of a *Babesia* sp. infection in the United States in a white-tailed deer (*Odocoileus virginianus*) from New Mexico. The first isolation of a *Babesia* sp. in white-tailed deer in Texas (USA) was reported by Emerson and Wright (1968). This organism was subsequently named *Babesia odocoilei* (Emerson and Wright, 1970) and has been described from deer in the pineywoods of eastern Texas (Robinson et al., 1968; Emerson, 1969) but it has not been reported on the Edwards Plateau (Emerson, 1969) or the central Texas coast of the Gulf of Mexico (Glazner and Knowlton, 1967). Perry et al. (1985) isolated *B. odocoilei* from the blood of white-tailed deer collected in the Great Dismal Swamp in Virginia (USA). The vector of this hematozoan is unknown although the lone star tick (*Amblyomma americanum*) has been implicated (Emerson, 1969). The indirect fluorescent antibody test has been used in the diagnosis of bovine babesiosis (Todorovic and Long, 1976) and in the detection of antibodies against *Babesia divergens* in red deer (*Cervus elaphus*) (Latif and Adam, 1973) and *Babesia capreoli* in roe deer (*Capreolus capreolus*) (Blancou, 1983). Isolation of *B. odocoilei* has been accomplished by subpassage of infective blood into a susceptible animal (Emerson and Wright, 1968; Perry et al., 1985) and by in vitro cultivation of separated erythrocytes from an infected deer (Holman et al., 1988). Seroepidemiological studies of bovine babesial infections have discussed the levels of inoculation rates that are present in enzootically stable and epizootically instable situations (Mahoney and Ross, 1972; Teclaw et al., 1985b). Inoculation rates are estimated from the prevalence of positive serology and the median age of the population sampled. These studies have shown that the level of the inoculation rate has a positive correlation with the risk of disease to that population. The objectives of this study were (1) to determine the prevalence of serological reactors to *Babesia odocoilei* in white-tailed deer populations at selected sites throughout Texas and Oklahoma by means of the indirect fluorescent antibody test, (2) to confirm the presence of the organism by direct isolation at selected sites, (3) to compare the prevalence rates of different age classes of white-tailed deer at a site with a high prev-

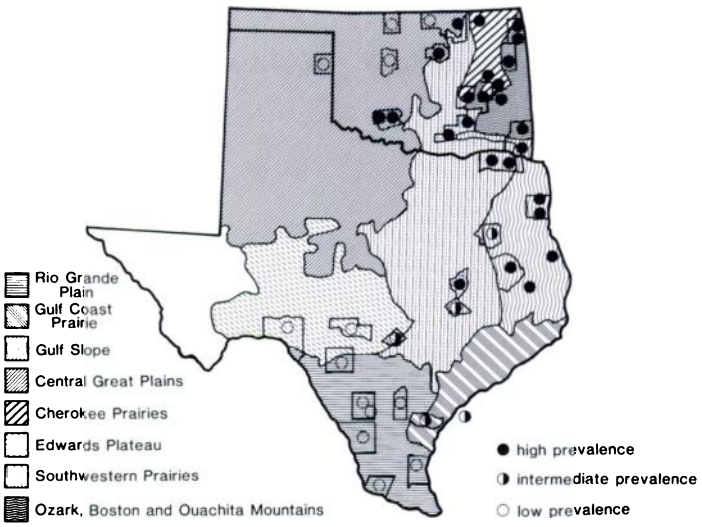

FIGURE 1. The prevalence of seroreactors in populations of white-tailed deer (*Odocoileus virginianus*) in different geographic areas of Texas and Oklahoma to *Babesia odocoilei* using the indirect fluorescent antibody test.

alence of positive antibody activity, and (4) to compare the calculated inoculation rates from data collected at selected sites.

MATERIALS AND METHODS

Sites were selected on the basis of accessibility to deer and location within different ecotypic areas of both states (Fig. 1). Wherever possible, the age of individual deer was estimated by tooth replacement and wear (Severinghaus, 1949). Sera were collected from individual deer, labeled and frozen at −20 C until tested. Each serum sample was thawed at room temperature and diluted 1:80 with phosphate-buffered saline. Control samples consisted of serum from (1) a deer known to be free of any hematozoan infection and (2) a second deer which had been infected with *B. odocoilei* culture material (Holman et al., 1988), but which was free of any other hematozoan infection. The method used for the indirect fluorescent antibody test was essentially that described by Todorovic and Long (1976). The antigen for the serologic test was prepared from thin smears of *B. odocoilei*-infected erythrocytes from in vitro culture (Holman et al., 1988). The conjugate used was a commercial fluoresceinated goat anti-bovine globulin serum (Cappel Products, Organon Teknika, Malvern, Pennsylvania 19355, USA). all tests were observed under 1,000× magnification using glycerine/phosphate-buffered saline medium with an AO model 120 microscope (American Optical, Scientific Instrument Division, Buffalo, New York 14215, USA) with an AO model 2071 epifluorescent unit which filtered the ultraviolet spectrum to a wavelength of 490 nanometers. Prevous experiments in our laboratory have demonstrated that sera from *Anaplasma marginale* or *Theileria cervi*-infected deer do not cross-react with the *B. odocoilei* antigen used in this test. Direct isolations of *B. odocoilei* were accomplished either by in vitro isolation and growth (Holman et al., 1988) or by sub-inoculation of pooled blood taken from several deer at a given site into a susceptible deer. The susceptibility of the recipient deer was enhanced by pretreatment with dexamethasone (Azium®, Schering Corporation, USA, Kenilworth, New Jersey 07033, USA) (Hussein, 1984) or by splenectomy (Kuttler et al., 1967b). Identification of hematozoan parasites observed in recipient deer was done by morphology of the parasite in Giemsa stained blood smears, using specific reference antiserum in an indirect fluorescent antibody test, or additional in vitro isolation and maintenance.

Prevalence rates were determined as a percentage of seropositive individuals divided by the total number of samples from a given site. Prevalence rates were grouped as high, intermediate or low relative to chi-square analysis (see Results for method). The chi-square test ($P = 0.05$) was used to compare the prevalence rates of different age classes from one site which had a high prevalence rate of positive serology to *B. odocoilei* and the greatest number of samples. The data from one site within each group

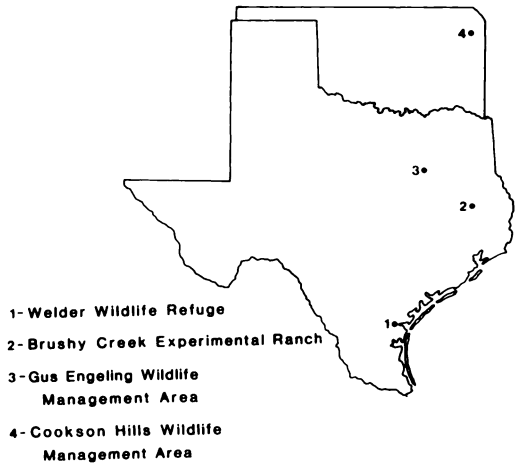

FIGURE 2. The locations in Texas and Oklahoma where *Babesia odocoilei* was directly isolated from white-tailed deer (*Odocoileus virginianus*).

which contained the greatest number of samples were used to calculate the respective inoculation rates. The inoculation rate (h) was derived from the equation $I = 1 - e^{-ht}$, where I is the prevalence of positive serological reactors, t is the mean age in days of the animals tested and e is the base of the natural logarithms (Mahoney and Ross, 1972). Designation of ecotypic areas was done from United States Department of Agriculture sources (Anonymous, 1965).

RESULTS

Five hundred eighty-one white-tailed deer serum samples were collected at 22 sites in six ecotypic areas in Texas (Fig. 1). There were no serologically positive reactors in the Central Great Plains (0/8). The prevalence of seropositive reactors for the remaining areas were as follows: 4% (4/92) for the Edwards Plateau, 2% (4/198) for the Rio Grande Plain, 51% (46/90) for the Gulf Coast, 84% (89/106) for the Gulf Slope, and 57% (54/95) for the Southwestern Prairie (Table 1). One hundred twenty-four white-tailed deer serum samples were collected from 20 sites in four ecotypic areas in Oklahoma. The prevalence rates for seropositive reactors were: 53% (20/38) for the Central Great Plains, 84% (31/37) for the Southwestern Prairie, 77% (20/26) for the Ozark, Boston, Ouachita Mountains and 83% (19/23) for the Gulf Slope (Table 2). The statistical comparison of the prevalence rates of all the sites in Texas and Oklahoma revealed significant differences which segregated into three groups. There were no significant differences among rates >55%, between 25% and 55%, and <25%. Therefore, prevalence rates greater than 55% were classified as high, prevalence rates >25% but <55% were classified as intermediate, and prevalence rates <25% were classified as low (Table 3). Direct isolations of *B. odocoilei* were accomplished at four sites in Texas and Oklahoma (Fig. 2). These sites were: (1) the Welder Wildlife Refuge on the central Gulf Coast of Texas (97°25'W, 28°06'N), (2) Brushy Creek Experimental Ranch in the Gulf Slope area of Texas (95°45'W, 31°00'N), (3) Gus Engeling Wildlife Management Area in the Southwestern Prairie of central Texas (96°26'W, 32°55'N), and (4) Cookson Hills Wildlife Management Area in the Ozark, Boston, Ouachita Mountain area of eastern Oklahoma (94°48'W, 35°41'N). The in vitro method of isolation was used only at Gus Engeling Wildlife Management Area in Texas. The method of isolation at the other sites was by the use of pooled blood collected at the respective site and inoculated into a susceptible recipient deer.

The comparison of prevalence rate against age was done with data collected from Brushy Creek Experimental Ranch due to the high prevalence rate of seropositive reactors, the confirmed presence of *B. odocoilei* at that site and the large number of samples obtained (Table 4). The prevalence rate of seropositive deer <12 mo of age was significantly less ($P = 0.05$) than the prevalence rates in all other age classes individually or collectively.

The inoculation rate (h) was calculated from the data collected from three sites in Texas, each from a high, intermediate, or low prevalence group. These sites were Brushy Creek Experimental Ranch (high), the Welder Wildlife Refuge (intermediate), and private ranches in Starr County along the Rio Grande River (low). These

TABLE 1. Geographic location and prevalence rates of white-tailed deer (*Odocoileus virginianus*) collected from Texas which were serologically positive to *Babesia odocoilei*.

Geographic location	Number of deer sampled	Number of seropositives	Prevalence
Central Great Plains			
Gene Howe Wildlife Management Area	8	0	0
Southwestern Prairie			
Caddo Wildlife Management Area	7	6	86
Gus Engeling Wildlife Management Area	16	8	50
Pat Mayse Wildlife Management Area	27	21	78
Somerville Wildlife Management Area	29	7	32
Robertson County	16	12	75
Edwards Plateau			
Robert Kerr Wildlife Management Area	43	0	0
Comal County	12	3	25
Uvalde County	34	1	3
Val Verde County	3	0	0
Gulf Slope			
Moore Plantation Wildlife Management Area	10	10	100
Harrison County	2	2	100
Panola County	10	9	90
Polk County	8	7	88
Brushy Creek Experimental Ranch	76	61	80
Gulf Coast			
Welder Wildlife Refuge	70	36	51
Matagorda Island Wildlife Management Area	20	10	50
Rio Grande Plain			
Chapparral Wildlife Management Area	31	0	0
James Daughtrey Wildlife Management Area	34	0	0
Kenedy County	49	3	6
La Salle County	10	1	10
Starr County	49	0	0
Webb County	25	0	0
Total	581	197	34

sites were chosen because of the large number of samples available from each. The calculated values of h were 0.0055 infective bites per day for the high prevalence site, 0.0007 infective bites per day for the intermediate prevalence site, and 0.0001 infective bites per day for the low prevalence site.

DISCUSSION

This study has extended the known distribution of *Babesia odocoilei* in white-tailed deer to include additional areas in Texas and Oklahoma both by serological diagnosis and direct isolation of the organism. The actual distribution of the parasite probably extends over a larger geographic area. This is the first report of *B. odocoilei* in Oklahoma. Glazner and Knowlton (1967) reported the presence of *Theileria cervi* in white-tailed deer at the Welder Wildlife Refuge but they did not observe *B. odocoilei*. This study confirms the presence of the parasite at that site as well. The absence or very low prevalence of antibody activity in some sections of the Great Plains, the Rio Grande Plain and the Edwards Plateau probably relates to the absence of an appropriate vector. Emerson (1969) reported that *B. odocoilei* was not

TABLE 2. Geographic location and prevalence rates of white-tailed deer (*Odocoileus virginianus*) collected in Oklahoma which were serologically positive to *Babesia odocoilei*.

Geographic location	Number of deer sampled	Number of positive samples	Prevalence
Central Great Plains			
Canton Wildlife Management Area	8	0	0
Great Salt Plains National Wildlife Refuge	3	0	0
Wichita Mountains National Wildlife Refuge	9	9	100
Fort Sill Military Reservation	8	7	88
Kay County	6	1	17
Payne County	4	3	75
Southwestern Prairie			
Atoka Wildlife Management Area	4	4	100
Pushmataha Wildlife Management Area	6	6	100
Fountain Head State Park	5	4	80
Tishomingo National Wildlife Refuge	10	8	80
Hughes County	7	4	57
Pittsburg County	5	5	100
Ozark, Boston, Ouachita Mountains			
Cookson Hills Wildlife Management Area	10	8	80
Spavinaw Wildlife Management Area	8	5	63
Fort Gibson State Park	5	4	80
Haskell County	3	3	100
Gulf Slope			
McCurtain County	2	2	100
Cherokee Prairie			
Cherokee Wildlife Management Area	4	4	100
Gruber Wildlife Management Area	9	8	89
Hulah Wildlife Management Area	8	5	63
Total	124	90	73

present in deer from the Edwards Plateau, and this study reaffirms that conclusion. Emerson (1969) also implicated the lone star tick as a vector of *B. odocoilei*. This tick is common in the Edwards Plateau area and the Rio Grande Valley. *Theileria cervi*, a blood parasite transmitted by this tick (Kuttler et al., 1967a), is present also in deer from that area and the Rio Grande Valley (Waldrup et al., 1989). Since *B. odocoilei* is not present in these deer populations, it seems less probable that *A. americana* is a vector of *B. odocoilei*.

The difference in the seropositive prevalence rate of deer <12 mo of age versus older deer is probably due to lack of exposure to the parasite or to the lag time beween exposure and detectable antibody activity. Antibody responses detected by the indirect fluorescent antibody technique can be demonstrated within 3 wk following inoculation of infective blood or in vitro culture material (K. A. Waldrup and G. G. Wagner, unpubl. data). It is possible that the vector-transmitted infection has a longer lag time, although the time from exposure to seroconversion with field tick transmission of bovine babesiosis is <1 mo (Teclaw et al., 1985a).

The calculated inoculation rates fit the mathematical models of bovine babesiosis as to the presence or absence of the parasite (Teclaw et al., 1985b). Sites demonstrating a high prevalence of positive seroreactors ($h > 0.005$) are probably enzootically stable with the indigenous deer population becoming infected at a young age when inverse age resistance factors are operative

TABLE 3. High, intermediate and low grouping of prevalence rates for seropositive reactors to *Babesia odocoilei* among white-tailed deer (*Odocoileus virginianus*) at selected sites in Texas and Oklahoma.

Location	Seropositive prevalence rate (%)
High prevalence sites	
Moore Plantation Wildlife Management Area (Texas)	100
Harrison County (Texas)	100
Wichita Mountains Wildlife Management Refuge (Oklahoma)	100
Atoka Wildlife Management Area (Oklahoma)	100
Pushmataha Wildlife Management Area (Oklahoma)	100
Haskell County (Oklahoma)	100
Cherokee Wildlife Management Area (Oklahoma)	100
McCurtain County (Oklahoma)	100
Panola County (Texas)	90
Gruber Wildlife Management Area (Oklahoma)	89
Polk County (Texas)	88
Fort Sill Military Reservation (Oklahoma)	88
Caddo Wildlife Management Area (Texas)	86
Brushy Creek Experimental Ranch (Texas)	80
Fountain Head State Park (Oklahoma)	80
Tishomingo National Wildlife Refuge (Oklahoma)	80
Cookson Hills Wildlife Management Area (Oklahoma)	80
Fort Gibson State Park (Oklahoma)	80
Pat Mayse Wildlife Management Area (Texas)	78
Payne County (Oklahoma)	75
Spavinaw Wildlife Management Area (Oklahoma)	63
Hulah Wildlife Management Area (Oklahoma)	63
Hughes County (Oklahoma)	57
Intermediate prevalence sites	
Welder Wildlife Refuge (Texas)	51
Gus Engeling Wildlife Management Area (Texas)	50
Matagorda Island Wildlife Management Area (Texas)	50
Somerville Wildlife Management Area (Texas)	32
Comal County (Texas)	25
Low prevalence sites	
Kay County (Oklahoma)	17
La Salle County (Texas)	10
Kenedy County (Texas)	6
Uvalde County (Texas)	3
Gene Howe Wildlife Management Area (Texas)	0
Robert Kerr Wildlife Management Area (Texas)	0
Val Verde County (Texas)	0
Chapparral Wildlife Management Area (Texas)	0
James Daughtrey Wildlife Management Area (Texas)	0
Starr County (Texas)	0
Webb County (Texas)	0
Canton Wildlife Management Area (Oklahoma)	0
Great Salt Plains National Wildlife Refuge (Oklahoma)	0

which allow for infection with little disease, similar to the epidemiology of endemic bovine babesiosis (Trueman and Blight, 1978). The sites with low prevalence of seropositive animals ($h < 0.0005$) are presumably not at risk since little transmission of the infection is occurring. The sites with an intermediate prevalence of seropositive reactors ($0.005 > h > 0.0005$) may be subject to disease in older animals if *B. odocoilei* is similar to *B. bovis* and *B. bigemina*. If the bovine babesiosis model is applicable, an intermediate prevalence rate of seropositive reactors indicates that the parasitic infection is being transmitted to enough individual animals within the indigenous population to maintain the infection in the vector population, but not all of the animals are being infected at a time when the inverse age resistance mechanisms are operating. Some older individual animals may become infected and exhibit some form of the disease. This disease may be difficult to ascertain in free-ranging populations. Hemolytic disease has been described in immunocompromised deer (Emerson and Wright, 1968; Perry et

TABLE 4. The comparison of different age classes of white-tailed deer (*Odocoileus virginianus*) from Brushy Creek Experimental Ranch (Trinity County, Texas) and serologic reactivity to *Babesia odocoilei* using the indirect fluorescent antibody technique.

Age class (yr)	Number of deer sampled	Number of deer seropositive	Prevalence (%)
<1 mo	18	8	44*
1–2	16	14	88
2–3	15	14	93
3–4	9	9	100
4–5	6	6	100
>5	8	6	75

* Significantly different from other age classes by chi-square analysis at $P \leq 0.05$.

al., 1985), but the true disease impact of this parasite on wild populations has not yet been explored. Until such research establishes the natural morbidity and mortality of this infection, managers should exercise caution in the practice of introducing mature white-tailed deer from areas of low prevalence (i.e., the Rio Grande Plain) to areas of high prevalence (i.e., the Southwestern Prairie). If older, introduced animals are at significant risk of disease due to babesiosis, then such introductions may have little additive effect on local populations.

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