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PREVALENCE OF *LEPTOSPIRA* ANTIBODIES IN WHITE-TAILED DEER, CADES COVE, GREAT SMOKY MOUNTAINS NATIONAL PARK, TENNESSEE, USA

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ABSTRACT: We conducted a study of the population dynamics, movement, and diseases of white-tailed deer (*Odocoileus virginianus*) in Cades Cove, Great Smoky Mountains National Park, Tennessee (USA) from 1980 to 1984. During the study 590 blood samples were collected from 518 deer, with some deer recaptured one or two times. The estimated percent of the herd sampled each year ranged from 8% to 28%. We also collected serum samples from 56 cattle pastured in Cades Cove.

Deer and cattle sera were tested using the microagglutination test for the presence of antibody to the following serovars of *Leptospira*: *pomona, hardjo, grippotyphosa, icterohemorrhagiae*, and *canicola*. One hundred and six deer (21%) were seropositive for only one of the serovars. We found that 57 (11%) of the deer had antibodies to serovar *hardjo*, 33 (6%) were positive for antibodies to serovar *pomona*, 15 (3%) were positive for antibodies to serovar *icterohemorrhagiae*, and one deer had antibodies to serovar *canicola*.

Age class and sex of deer were associated with antibody presence. Adult (≥1.5 yr) male deer were more likely to have antibodies than the other age class and sex groups ($P = 0.001$).

In recaptured deer, similar titers were found in samples from one deer taken 807 days apart. Titer declined below the screening dilution level (1:250) after 37 days in one deer.

Key words: White-tailed deer, *Odocoileus virginianus*, leptospirosis, prevalence, microscopic agglutination test, serological survey.

INTRODUCTION

The Great Smoky Mountains National Park (USA) is the most visited park in the national park system (U.S. Department of Interior, 1982). Within the Park, Cades Cove is one of the most visited areas. A major reason people give for visiting the Cove is the opportunity to view wildlife. White-tailed deer (*Odocoileus virginianus*) are one of the most prized species available for viewing by visitors (Hastings, 1986). Because of the intrinsic value of this wildlife resource, any situations or events that might jeopardize the health or status of this deer population are of concern.

Because most major predators of deer have been extirpated from the Park and because hunting is not allowed, major pressures that control deer populations are missing. Consequently, the density of the herd is one of the highest recorded in the Southeast at 0.38 deer/hectare by spotlight count and 0.23 deer/hectare by mark/recapture population estimates (Wathen and New, 1989). The potential for an epizootic was illustrated in 1971 when hemorrhagic disease occurred among deer in the Cove (Fox and Pelton, 1973).

The deer population recovered from the epizootic and the density continued to increase in the 1970's (Burst and Pelton, 1978; Kiningham, 1980). Based on accumulating information about the impact of the deer on vegetation, population genetics, and body condition status of the herd, concerns were raised over the density of the herd as well as the potential for another epizootic. Consequently, personnel of the Resources Management Division of the Park in cooperation with the Tennessee (USA) Wildlife Resources Agency initiated a series of deer removals from the Cove in 1981. Many captured deer were translocated to other parts of east Tennessee as part of a deer restoration program.

We initiated this study in 1980 to assess

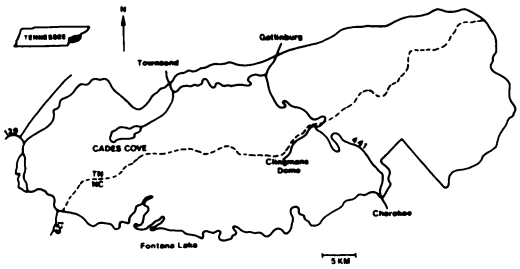

FIGURE 1. Great Smoky Mountains National Park (Hastings, 1986).

TABLE 1. Number (%) of 518 white-tailed deer positive[a] by the microagglutination test for four serovars of *Leptospira interrogans*, Cades Cove, Great Smoky Mountains National Park, Tennessee, 1980 to 1985.

Serovar			
hardjo	*pomona*	*ictero-hemorrhagiae*	*canicola*
57 (11.0)	33 (6.4)	15 (2.9)	1 (0.2)

[a] Deer were considered positive for a specific serovar if serum was positive (≥1:250) only for that serovar.

the status and potential impact of infectious diseases on the Cades Cove deer herd. We focused on hemorrhagic disease, leptospirosis, bovine virus diarrhea, infectious bovine rhinotracheitis, anaplasmosis and brucellosis. Here, we report our findings on leptospirosis, caused by *Leptospira* spp.

MATERIALS AND METHODS

Cades Cove is bounded by 35°36′N and 80°46′ to 83°51′W (Fig. 1). During the 5 yr of the study, we collected 590 serum samples from 518 deer in the Cove. Most samples came from captured animals. Deer were captured in Stephenson traps and cannon nets (Halls, 1984), and by chemical immobilization. Immobilizing drugs were delivered using a Pneu-dart .50 caliber rifled dart gun (Pneudart, Inc., Williamsburg, Pennsylvania, USA) with .22 caliber charges (Speed Fastener, Inc., St. Louis, Missouri, USA). Immobilizing drugs included succinylcholine chloride (Anectin, Pneudart, Inc., Williamsburg, Pennsylvania) at a dose range of 5 to 9 mg per animal depending on size. When succinylcholine chloride was used, oxygen or an Ambubag (Ohmeda, Orchard Park, New York, USA) was available in case resuscitation was necessary. In addition, some deer were immobilized using a combination of xylazine hydrochloride (Rompun, Bay Vet Division, Miles Laboratories, Shawnee Mission, Kansas, USA) and atropine sulfate (Am-Vet Pharmaceuticals, Ft. Collins, Colorado, USA). Dosage ranged from 160 to 240 mg xylazine and 10 to 15 mg atropine per animal depending on size. This combination was delivered using Pneu-dart CL type 1 cc liquid darts. Many deer were translocated after capture to parts of Tennessee where small populations had been documented. Others were released after recovery at the capture site. The recapture of some of these deer allowed duplicate sampling of some individuals.

Post-mortem samples were collected from a few deer that had been killed recently by vehicles or shot for abomasal parasite counts. Leptospire culture was not attempted from the few deer available for necropsy.

Once a deer was immobilized, it was given a general physical examination, a numbered ear tag was attached, and age was estimated using the method of Severinghaus (1949). We relied on tooth eruption as the primary means of age estimation for the first few years; however, tooth wear was used to age the deer after the first few years. Because tooth wear is more subjective than eruption and a variety of people were aging deer (people with varying experience in aging), we collapsed ages into two age classes, adults (≥1.5 yr) and subadults (<1.5 yr). We recorded sex and location of capture, and collected approximately 40 to 50 ml of blood from the jugular vein. Some blood was collected in tubes with ethylene diamine tetraacetic acid (EDTA) but most of each sample was allowed to clot. After clotting, we separated the serum and froze it until testing.

We also collected 56 blood samples during winter 1983 and spring 1984 from cattle pastured in the Cove. These samples were collected when the cattle were being handled for other reasons such as loading for market. Based on two herd counts conducted in 1983 and 1984 these samples represent approximately 13% of the herd. Thorough physical exams were not conducted at the time of blood collection but no gross signs of illness were noted. The herd is a mixed breed herd but the owner uses Angus bulls primarily. Leptospirosis vaccine was not used in the herd prior to this study and no animals had entered the herd except by birth for at least 10 yr prior to this study. The owner sells young stock (usually about 2 yr of age) when the market is favorable. The herd is geographically isolated with the next closest cattle being 5 km away over a mountain range.

We tested the serum samples from the deer and cattle for the presence of antibodies to five leptospiral serovars (*pomona, hardjo, grippotyphosa, canicola, icterohemorrhagiae*). Testing was done at the C. E. Kord Diagnostic Lab-

TABLE 2. Frequency (%) of white-tailed deer positive by the microagglutination test for *Leptospira interrogans* antibody by age class and sex, Cades Cove, Great Smoky Mountains National Park, Tennessee, 1980 to 1985.

	Age class					
	<1.5 years		≥1.5 years		Total	
Sex	Frequency	Percent	Frequency	Percent	Frequency	Percent
Male	5/24[a]	9.3	43/115	37.4	48/169	28.4
Female	5/64	7.8	54/230	23.5	59/294	20.1
Total	10/118	8.5	97/345	28.1	107/463	23.1

[a] Number of positive deer in age class, sex category/number of deer sampled in the same age class, sex category. Data sheets on 55 deer were missing; age or sex data were not used.

oratory, Nashville, Tennessee (USA), using the microagglutination test (MAT) (Faine, 1982) to screen the sera at a dilution of 1:250. We considered a deer positive for a specific serovar if serum was positive (≥1:250) only for that serovar. We used the Epi Info Analysis program (Dean et al., 1990) to compare the age class and sex as separate variables in relation to antibody presence. We used the FUNCAT statistical program (SAS Institute, Inc., 1982) to evaluate the combined effect of the age class and sex variables.

RESULTS

We found 106 deer (21%) seropositive (≥1:250) for only one serovar (Table 1). In some instances, age and sex were not recorded at the time of darting. Only deer with recorded age and sex were used for statistical analysis (Table 2). Fifty-four deer (10%) were recaptured, nine of which were positive for specific serovars (Table 3).

Based on the Epi Info Analysis program, males were more likely to be seropositive than females but the difference was not statistically significant (odds ratio = 1.53, Cornfield 95% confidence limits 0.96 < odds ratio (OR) < 2.44, Chi-square = 3.64, $P = 0.06$). Adults were more likely to be seropositive than subadults (odds ratio = 4.22, Cornfield 95% confidence limits 2.04 < OR < 8.97, Chi-square = 19.1, $P = 0.00001$). Using the FUNCAT statistical program, adult males were more likely to be seropositive than females of any age or subadult males ($P = 0.001$). Based on physical examinations no clinical disease was observed during the study period which could be attributed to leptospirosis.

Seropositive deer were found in all parts of the Cove (Fig. 2). The site of darting was not recorded or incompletely recorded on 63 of the 590 data sheets.

Among the 56 cattle tested, no antibody was found to serovars *grippotyphosa*, *icterohemorrhagiae*, or *canicola*. Two animals had antibody titers only to serovar *pomona* (1:800 and >1:3,200). Twenty-eight (50%) of 56 animals were positive for antibodies only to serovar *hardjo*. Ten cattle had titers to serovar *hardjo* of >1:3,200, 11 had titers of 1:1,600, five had titers of 1:800, and two had titers of 1:400.

DISCUSSION

Descriptions of clinical signs of leptospirosis in white-tailed deer are based on limited experimental infections with serovar *pomona* only. Other than transient fever, some deer exhibited anorexia, weakness, anemia, hemoglobinuria, icterus and death (Ferris et al., 1960; Trainer et al., 1961; Reilly et al., 1962a; Roth, 1962, 1970; Shotts, 1981a, b). Abortion has resulted from experimental infection and has been documented in at least one naturally infected deer (Trainer et al., 1961; McGowan et al., 1963). Lesions often are limited to interstitial nephritis in naturally occurring infections (Abdulla et al., 1962; Roth et al., 1964). Experimental infections also have resulted in hepatitis and hemorrhages (Ferris et al., 1960; Roth, 1970).

The most extensive studies of leptospirosis in white-tailed deer have been sero-

TABLE 3. Recaptured white-tailed deer positive by the microglutination test for *Leptospira interrogans*, Cades Cove, Great Smoky Mountains National Park, Tennessee, 1980 to 1985.

Serovar	Deer identification number	Capture dates (mo-day-yr)	Reciprocal titer	Days between first and second capture
hardjo	193	9-27-82	1000	807
		12-12-84	500	
	436	6-27-84	250	163
		12-7-84	250	
	458	8-22-84	250	107
		12-7-84	250	
pomona	42	9-15-80	500	449
		12-8-81	500	
	211	9-30-82	500	75
		12-14-82	250	
	455	8-7-84	250	127
		12-12-84	<250	
	468	10-17-84	500	56
		12-12-84	<250	
	472	11-1-84	250	36
		12-7-84	<250	
icterohemorrhagiae	300	6-23-83	250	77
		9-8-83	<250	

logic surveys. The earliest surveys were conducted in the late 1950's and the 1960's. Antibodies to serovars *grippotyphosa* and *pomona* are commonly reported in white-tailed deer (Shotts, 1981a, b). This could be a reflection of the most common leptospires infecting deer, or reflect an emphasis of serologic surveys on the serovars important to domestic livestock. The latter case could be true especially of serovar *pomona*.

Although positive results at 1:100 to 1:200 serum dilutions with the microagglutination test generally are considered significant and of diagnostic importance (Diesch et al., 1976; Shotts, 1976; Hanson, 1982), we believed that by increasing the screening dilution to 1:250, we could determine the probable identity of infecting serovar(s). Paradoxical reactions are not uncommon in serological studies using several antigens. In such reactions titers to several serovars may be recorded that are within twofold dilutions of each other (Shotts, 1976). Consequently, we considered a deer positive for a specific serovar if serum was positive (≥1:250) only for that serovar.

Reactions to serovar *pomona* have been reported in three serologic surveys (Andrews et al., 1964; Shotts and Hayes, 1970; Harrington, 1975) with 43.0%, 52.1% and 22.6% of deer positive, respectively. Microagglutination tests with screening dilutions of 1:100 or greater also were used in these surveys. Antibodies to serovar *pomona* were found in 37% of deer by Reilly et al. (1962b) who used latex agglutination and complement fixation tests. Trainer and Hanson (1960) found 28% of deer sera positive using the plate agglutination and the agglutination-lysis technique. In our study, serovar *pomona* antibody was found in 6% of all deer sampled.

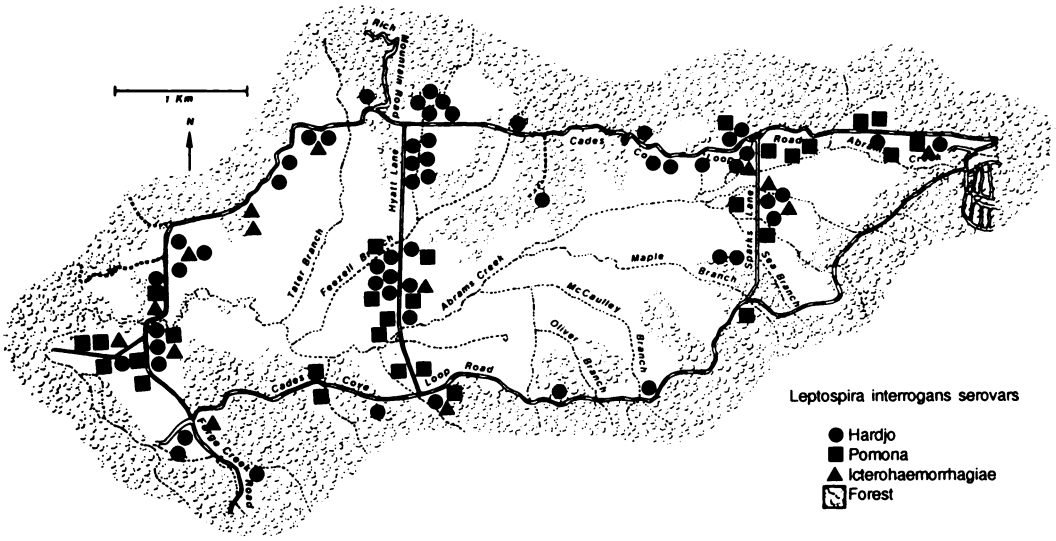

FIGURE 2. Approximate darting locations of white-tailed deer seropositive for three serovars of *Leptospira interrogans* (≥1:250 titer by the microagglutination test to only one serovar), Cades Cove, Great Smoky Mountains National Park, 1980 to 1985.

Haugen (1967), using the microscopic agglutination test, specifically reported finding serovar *hardjo* titers in 1.1% of 369 deer sera collected in Iowa. Goyal et al. (1992) failed to find evidence of antibody for serovar *hardjo* in 204 serum samples from 124 deer using a microscopic agglutination test.

Serovar *hardjo* is associated strongly with cattle. In the United States, serovar *hardjo* has been isolated from cattle and humans (Hanson, 1981), but not from a wildlife host (Diesch et al., 1976; Hanson, 1982). In most cases, serovars *hardjo* and *pomona* apparently are transmitted directly among cattle whereas other serovars infecting cattle likely are from wildlife (Diesch et al., 1976). Leptospires often exhibit a species preference and cattle are considered the natural carrier host of serovar *hardjo* (Diesch et al., 1976; Hanson, 1982; Blood et al., 1983). Cattle can shed leptospires in the urine for >1 yr under experimental conditions (Thiermann, 1982).

Positive titers to serovar *hardjo* are the most common serologic finding in cattle in some parts of the U.S. (White and Sulzer, 1982; Blood et al., 1983; Diesch, 1983). There is also evidence that serovar *hardjo* infection is rather widespread across the country. Of 66,522 cattle sera collected from 18 states, 7.2% were serovar *hardjo* reactors; by comparison, 6.5% were reactors to serovar *pomona* (Diesch et al., 1976).

The location of 56 of 57 serovar *hardjo*-positive deer, 29 of 33 serovar *pomona*-positive deer and 14 of 15 serovar *icterohemorrhagiae*-positive deer are known (Fig. 2). In addition, one deer (location not shown) darted approximately midway along Spark's Lane was positive for serovar *canicola*. Spark's Lane transects pastures used permanently for cattle as does Hyatt Lane at its southern end. Exposure potential for deer may be increased during the winter as they utilize hay put out for cattle and are perhaps drawn to salt blocks. This would increase their contact with areas contaminated by cattle urine.

Antibody titer to serovar *hardjo* in one deer declined from 1:500 to below 1:250 within 392 days; however, three deer captured twice were positive for serovar *hardjo* each time they were captured (Table 3). A titer to serovar *hardjo* was detected in sera collected from the same deer 807 days apart. This could represent a persistent titer or reexposure to the stimulating

antigen. In two other studies, *Leptospira* antibody titers in deer did not persist long, compared to cattle (Trainer et al., 1961; Goyal et al., 1992).

Three deer, captured twice, initially were positive for serovar *pomona* (1:250 for two deer and 1:500 for one deer). However, when recaptured, titers had declined below the screening level within 127, 56, and 36 days, respectively. Sera from two recaptured deer were positive for serovar *pomona* each time they were captured (Table 3). One deer captured twice was positive for serovar *icterohemorrhagiae* (1:250) but the titer had dropped below the screening level 77 days later.

The amount of serum available for testing for leptospiral antibodies was limited because each serum sample had to be divided among other investigators for testing for antibody to other infectious agents and selected chemical parameters (Dlutkowski, 1985; Wathen and New, 1989). We also wanted to determine end point titers up to 1:3,200 for positive sera. Consequently, because of the limited volume of serum and laboratory resources, we were unable to test for antibodies to serovars other than those already established in the laboratory.

It is unknown whether leptospirosis causes illness or death in white-tailed deer. Most infections probably are mild clinically. The infertility and lactation problems documented in cattle from serovar *hardjo* infection have not been documented in deer. Consequently, the ramifications of leptospirosis infection to the Cove deer herd appear minimal. Based on our serologic survey, serovar *hardjo* is the predominant serovar infecting deer in the Cove; however, culture is necessary in order to definitively identify infecting serovars. This question deserves further investigation.

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