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BORRELIA SP. INFECTION IN COYOTES, BLACK-TAILED JACK RABBITS AND DESERT COTTONTAILS IN SOUTHERN TEXAS

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ABSTRACT: Coyotes (*Canis latrans*) from southern Texas were sampled for antibodies to *Borrelia burgdorferi* from 1980 to 1986; black-tailed jack rabbits (*Lepus californicus*) and desert cottontails (*Sylvilagus audubonii*) were sampled in 1986. Coyote fetuses, adult coyote kidneys, and black-tailed jack rabbit and desert cottontail kidneys were cultured for *B. burgdorferi* in 1986. Results of indirect immunofluorescent antibody (IFA) tests for *B. burgdorferi* in coyotes were as follows (number positive at a dilution of ≥1:128/number tested): 1980 (0 of 30), 1981 (0 of 21), 1982 (0 of 53), 1983 (0 of 78), 1984 (47 of 97), 1985 (20 of 88), and 1986 (42 of 80). Eight of 26 black-tailed jack rabbits and two of seven desert cottontails tested in 1986 had IFA titers to *B. burgdorferi* of ≥1:128. *Borrelia burgdorferi* was isolated from one of five coyote fetuses, three of 31 adult coyote kidneys, and two of 10 black-tailed jack rabbit kidneys in 1986. These results indicate that *B. burgdorferi* infection has been present in coyotes in Texas, at least since 1984 and that transplacental transmission occurs.

Key words: *Borrelia burgdorferi*, coyote, *Canis latrans*, black-tailed jack rabbit, *Lepus californicus*, desert cottontails, *Sylvilagus audubonii*, transplacental transmission, Lyme disease, survey.

INTRODUCTION

Lyme borreliosis (a spirochetal tick-borne zoonosis) was first reported in the United States in 1969 in a Wisconsin hunter (Scrimenti, 1970). In 1975, the infection was given the name Lyme disease because of an outbreak of arthritis caused by the infection in children from Lyme, Connecticut (Steere et al., 1978). Infection with the causative spirochete (*Borrelia burgdorferi*) can occur in domestic animals, including dogs (Burgess, 1986), horses (Burgess et al., 1986), cows (Burgess et al., 1987) and wild animals (Bosler et al., 1984). The white-tailed deer (*Odocoileus virginianus*) and the white-footed mouse (*Peromyscus leucopus*) are the principal vertebrate reservoirs of the spirochete (Bosler et al., 1984; Levine et al., 1985). The primary tick vectors of the disease in the United States are *Ixodes dammini* (Burgdorfer et al., 1982) and *I. pacificus* (Burgdorfer et al., 1985). Lyme disease was first reported from Texas in 1984 (Rawlings, 1986). Antibodies to *B. burgdorferi* were reported from two of 10 black-tailed jack rabbit (*Lepus californicus*) sera from western Texas in 1985 (Rawlings, 1986) suggesting that wild mammals from Texas may be infected with *B. burgdorferi*. The objective of this study was to determine if coyotes (*Canis latrans*), black-tailed jack rabbits and desert cottontails (*Sylvilagus audubonii*) from southern Texas were infected with *B. burgdorferi*.

MATERIALS AND METHODS

Serum samples

Coyote sera samples were collected from fall 1980 to fall 1986 during ongoing research on coyote ecology in southern Texas (Denver Wildlife Research Center, Animal Plant Health Inspection Service, United States Department of Agriculture, 319 Stowe Street, Laredo, Texas 78041, USA). Coyotes (≥ 5-mo-old) were trapped on eight sites of 3,000 to 8,000 ha in Webb County, Texas, (27°50′N, 99°20′W) within 60 km north and east of Laredo in fall (October to November) 1980 through 1982, spring (March to April) 1983, and spring (February to June) and fall 1984 through 1986. The coyotes range over this entire area and are considered one population. Coyotes at two of the sites within this area were sampled on different years (27°45′N, 98°54′W in 1981 and 1985; 27°19′N, 99°19′W in 1980 and 1984). Jack rabbits and cottontails were shot in April and September

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1986. Whole blood was extracted with vacutainers from the brachial and femoral veins of coyotes that were marked and released and by cardiac puncture from jack rabbits, cottontails and coyotes that were sacrificed. In 1986, coyote and lagomorph kidneys and coyote fetuses were removed immediately after death. Whole blood was maintained at a cool temperature until delivery to the laboratory where it was centrifuged and the serum was decanted. Sera, kidneys and fetuses were frozen and shipped to the University of Wisconsin School of Veterinary Medicine (2015 Linden Drive, Madison, Wisconsin 53706, USA)

Indirect immunofluorescent antibody test (IFA)

Serum samples were tested for antibodies to *B. burgdorferi* by the IFA test using standard procedures (Burgess, 1985) and fluorescein conjugated goat anti-canine IgG serum for the coyote samples and fluorescein conjugated goat anti-rabbit IgG serum for the lagomorph sera (Cappel Laboratories, Cooper Biomedical, West Chester, Pennsylvania 19380, USA). Hyperimmune sera from experimentally infected dogs and rabbits were used for positive control sera and sera from laboratory reared dogs and rabbits were used as negative control sera. Two-fold dilutions of the sera from 1:2 to 1:4,096 were prepared. The IFA end point was the highest dilution to have specific fluorescence of the spirochetes. Ten of the sera were also tested by IFA for antibodies to *B. hermsii* (obtained from R. Johnson, Department of Microbiology, University of Minnesota, Minneapolis, Minnesota 55455, USA) which is another *Borrelia sp.* found in Texas (Burgdorfer, 1985) to determine if there was any cross-reactivity.

Culturing of tissue samples for *Borrelia burgdorferi*

Kidney samples from the coyotes, jack rabbits and coyote fetuses obtained from the 1986 coyotes were ground with a mortar and pestal and a 10% suspension made in BSK 11 media without antibiotics (Johnson et al., 1984). A 0.1 ml sample of the suspension was placed in 7 ml of BSK 11 media and incubated at 34 C. A drop from each culture tube was placed on a slide and examined once weekly by dark field microscopy for 6 wk for the presence of spirochetes. Spirochetes were identified by staining with the *B. burgdorferi* specific mouse monoclonal antibody H5332 (obtained from E. Bosler, New York State Department of Health, Stony Brook, New York 11794, USA) (Barbour et al., 1983) followed by fluorescein labelled goat anti-mouse IgG serum (Cappel Laboratories, West Chester, Pennsylvania 19389, USA). Slides with the eighth passage *B. burgdorferi* isolate from *P. maniculatus* were used as positive controls. Frozen kidneys were also sectioned at 7 μm on a microtome and the sections placed on slides. The sections were then stained with the H5332 monoclonal antibody as described above.

RESULTS

The results of IFA testing for *B. burgdorferi* antibodies in coyote sera are shown in Table 1. In the years 1980 (30 samples), 1981 (21 samples), 1982 (53 samples) and 1983 (78 samples) the antibody titers were all <1:128. In 1984 47 of 97 sera had antibody titers ranging from 1:128 to 1:4,096, in 1985 20 of 88 sera had antibody titers from 1:128 to 1:1,024 and in 1986 38 of 80 sera had antibody titers from 1:128 to 1:1,024. Results of IFA tests for *B. burgdorferi* antibodies in jack rabbits in 1986 were eight of 26 at a titer of ≥1:128 (three at 1:128, two at 1:256, two at 1:512, and one at 1:1,024). Cottontails had two of seven sera with titers of 1:128. Positive cultures for spirochetes were one of five fetal coyote kidneys (positive fetus was from a seronegative female), three of 31 adult coyote kidneys (two seronegative, one serum titer 1:512), two of 10 jack rabbit kidneys (one seronegative, one serum titer 1:1,024) and zero of five cottontail kidneys. The spirochetes did multiply in the culture medium as the numbers in each tube increased over time and dividing spirochetes could be seen. These spirochetes could be subcultured but the numbers of spirochetes were few. The cultures were all contaminated with bacteria because the kidneys had been taken in the field and sterile technique was difficult to maintain. The spirochetes could not be separated from the bacteria and did not grow to sufficient numbers to be able to identify the strain. They did have typical *Borrelia sp.* morphology. One of the culture positive adult coyote kidneys also had spirochetes that fluoresced with the H5332 monoclonal antibody on stained frozen kidney sections identifying the spirochete as *B. burgdorferi*. The results of comparison of IFA titers for *B. burgdorferi* and *B. hermsii* are shown on Table 2. Only four of the 10

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TABLE 1. Results of coyote sera tested by the indirect immunofluorescent antibody assay for *Borrelia burgdorferi* antibodies from 1980 to 1986. Antibody titers are given as the reciprocal of the end point dilution.

Year	*B. burgdorferi* antibody titer								Total number of samples
	0–8	64	128	256	512	1,024	2,046	4,096	
1980	26	4							30
1981	21								21
1982	49	4							53
1983	67	11							78
1984	39	11	8	4	8	19	4	4	97
1985	45	23	7	9	2	2			88
1986	17	21	16	2	6	18			80

samples tested had titers to *B. hermsii* of 1:128 while all 10 samples had antibody titers to *B. burgdorferi* of 1:512 or greater.

DISCUSSION

These findings show that *Borrelia sp.* (most probably *B. burgdorferi*) infection has been present in coyotes in Webb County, Texas since 1984 and that transplacental infection can occur in infected coyotes. It is not known how *B. burgdorferi* first arrived in Texas but apparently the infection in the southern part of the state appeared in wildlife and humans at about the same time (Rawlings, 1986). The positive identification of the kidney tissue spirochete and the low serum cross reactivity with *B. hermsii* (another *Borrelia* sp. found in the western United States; Barbour and Hayes, 1986) suggests that coyotes were infected with *B. burgdorferi*. There is some degree of cross-reactivity between *B. burgdorferi* and *B. hermsii* (DNA relatedness 58% according to Hyde and Johnson, 1984) but it is not 100% and sera with antibodies specific to *B. burgdorferi* should have a higher titer to *B. burgdorferi* than to *B. hermsii*. In previous studies, adsorption of sera from human patients with Lyme disease with *B. hermsii* decreased the *B. burgdorferi* titers by only two-fold (Craft et al., 1984) again indicating that titers of antibody specific to *B. burgdorferi* would be higher to *B. burgdorferi* than to *B. hermsii*. The large number of adult coyotes with titers ≥1:128 in 1984 and the absence of coyotes with titers ≥1:128 prior to 1984 indicates that *B. burgdorferi* infection was acquired in 1984 or late 1983. Experimentally infected dogs develop titers of ≥1:128 at 21 days post-infection and maintained high titers up to 100 days postinoculation (Burgess, 1986). Wildlife may be important in maintaining *B. burgdorferi* infection in Texas because the infection occurs in several species. Interaction of coyotes, jack rabbits and cottontails with livestock and dogs could bring infected ticks in close association with humans. *Borrelia burgdorferi* antibodies have been found in jack rabbits in California (Lane and Burgdorfer, 1988).

Transplacental infection of *B. burgdorferi* has been shown in humans, cows and

TABLE 2. Results of indirect immunofluorescent antibody tests on 10 coyote sera from 1986. Sera were tested for antibodies to *Borrelia burgdorferi* and *Borrelia hermsii*. Titers are given as the reciprocal of the end point dilution.

Coyote	Titer	
	B. burgdorferi	*B. hermsii*
1	1,024	8
2	1,024	64
3	4,096	8
4	2,048	8
5	1,024	64
6	1,024	128
7	1,024	8
8	1,024	128
9	512	128
10	1,024	128

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horses and has been associated with abortions and fetal mortality (Schlesinger et al., 1985; Burgess, 1988). The effect of transplacental infection in the coyote is unknown. The case of an antibody negative coyote having a *B. burgdorferi* culture positive fetus might suggest a localized infection in the reproductive tract or that the female was infected recently and had insufficient time to develop antibodies. Chronic infection of the reproductive tract with no antibody response occurs with other spirochete infections such as in animals infected with *Leptospira interrogans* serovar *australis* (Thiermann, 1984). This could mean that a survey for *B. burgdorferi* infection using the presence of antibodies alone as the method of detection may underestimate the prevalence of infection.

Oral infection with *B. burgdorferi* has been demonstrated experimentally in *Peromyscus* spp. (Burgess and Patrican, 1987) and infection by direct contact has been demonstrated in dogs and *Peromyscus* spp. in the absence of arthropod vectors (Burgess, 1986; Burgess et al., 1986). *Borrelia burgdorferi* has been isolated from the urine of cows and *Peromyscus* spp. Although prey-to-predator transmission has not been reported, that possibility exists. Coyotes may become infected from eating infected jack rabbits and cottontails. There are several tick species in Texas which could be involved in the transmission of *B. burgdorferi*. *Borrelia burgdorferi* has been isolated from the rabbit tick *Dermacentor parumapertus* from Texas (Rawlings, 1986). *Haemaphysalis leporispalustris* is commonly found on rabbits and *B. burgdorferi* has been isolated from this tick species (Anderson and Magnarelli, 1984). *Amblyomma americanum* and *Ixodes scapularis* have both been recovered from coyotes from Texas (Pence et al., 1981) and both of these tick species have been shown to be potential vectors for Lyme disease (Schulze et al., 1984; Burgdorfer and Gage, 1986). It is possible that several of these tick species may be transmitting *B. burgdorferi* in wildlife in Texas. Further work is needed in order to determine exactly what tick species are important vectors in the southwestern United States.

Dogs infected with *B. burgdorferi* can develop lameness, fever, anorexia and arthritis (Kornblatt et al., 1985). If coyotes develop similar signs it could adversely affect their populations by interfering with social interactions and predatory skills. If reproductive impairment occurs in coyotes, jack rabbits or cottontails it could reduce recruitment and may reduce population numbers. Further work is necessary in order to determine the impact of *B. burgdorferi* infection on the population ecology of these species.

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