Spirochete Seroprevalence in Rodents of North Central Texas

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ABSTRACT

Prevalence of exposure to spirochetes, particularly *Borrelia burgdorferi*, in rodents of North Central Texas was determined using an indirect immunofluorescent assay. The Chi-square test was used to analyze positive sera reaction across season and tick distribution across rodent species. This test did not detect a seasonal significant difference of seropositive specimens ($X^2 = 2.45$, df = 3, $P > 0.05$). Percent tick infestation was significantly different across rodent species ($X^2 = 14.176$, df = 7, $P < 0.05$). Other data collected included 1) seasonal distribution of seropositive reactions in adult rodents, 2) distribution between trapsites of adult rodent positive serological reactions, 3) tick infestation organized by individual rodent stage and sex, 4) percent of each species seropositive, and 5) percent adult seropositive reactions per month.

KEYWORDS: *Borrelia burgdorferi, Peromyscus leucopus*, lyme disease

Lyme borreliosis, caused by the spirochete *Borrelia burgdorferi*, is a health hazard throughout the United States. Antibodies to *Borrelia burgdorferi* have been detected in mammals throughout the United States (Godsey et. al., 1987; Magnarelli et al., 1984b). Rodents, mainly mice, in different regions of the country have been infected with the spirochete (Anderson et al., 1987a; Loken et al., 1985).

There are three known geographic foci of lyme borreliosis in the United States: 1) New York-Connecticut area, 2) California, and 3) the Wisconsin area. *Peromyscus leucopus* has proven to be a major reservoir of the disease along the Atlantic coast of North America (Donahue et al., 1987; Levine et al., 1985; Mather et al., 1989) and also in the midwest portion of the United States (Anderson et al., 1987b; Godsey et al., 1987).

In Texas, research has been conducted on prevalence of *Borrelia* spirochetes in ticks (Teltow et al., 1991; Rawlings and Teltow, 1994) and seroprevalence of antibodies to *Borrelia burgdorferi* in horses (Cohen et al., 1992). Teltow et al. (1992) isolated spirochetes from four species of arthropods--three ticks and a flea. However, they were only able to culture spirochetes from 0.68% of pooled samples

Julie Rawlings, Zoonosis Control Division, Texas Department of Health, Austin, provided the sera which was used as a positive control. Dr. Alan Barbour, University of Texas Health Sciences Center, San Antonio, provided the monoclonal antibodies used in this study. Dr. Jesse Cocke, Texas Agricultural Extension Service, Stephenville, assisted with identification of ticks. *Corresponding author.

of ticks and 1.4% of pooled flea samples. Rawlings and Teltow (1994) fared better, isolating spirochetes in 1.03% of individual adult *Amblyomma americanum* (L.)--the only tick found to harbor spirochetes during the course of their study.

Serological studies have been used to determine prevalence of *Borrelia* spirochete infection in mammals (Anderson et al., 1983; Magnarelli et al., 1984a, 1984b). A number of protocols for serological detection exist; one is the indirect immunofluorescent assay or IFA (Magnarelli et al., 1984b, 1984c; Russell et al., 1984). A disadvantage of this test is non-specificity. A positive reaction when using *B. burgdorferi* as the antigen for serum antibodies does not preclude the possibility of cross reaction if the mammal was exposed to another species of *Borrelia*.

Mammalian reservoirs of *B. burgdorferi* in Texas have not yet been determined. Cohen et al. (1992) reported that only 1 in 469 samples of horse serum collected from central Texas was positive for *B. burgdorferi* infection. If the epidemiology of the disease is similar to other areas of the United States, then rodents should be involved. It is likely that sylvatic mice, and possibly other small mammals are reservoirs of the disease in Texas. Thus, the objective of this study was to determine the prevalence of *Borrelia* exposure in native small mammals in North Central Texas.

MATERIALS AND METHODS

Selection of Subjects and Research Design

Trap areas were chosen to ensure sampling of *P. leucopus* (Schmidly, 1983). Rodents were trapped at six locations in North Central Texas (Fig. 1). Ten to twenty traps per site were open during the specified dates (reported below) except in inclement weather. Sympatric species trapped at these sites were also tested. Trapsite A was located seven miles east of Stephenville, Texas, in Erath County. This trapsite was sampled every month from 19 July 1989 until 19 July 1990. Trapsite B was located one mile northeast of Stephenville, Texas, in Erath County. Trapping was conducted from August through October 1989. Trapsite C was located six miles south of Weatherford, Parker County, Texas. Traps were set from September through November 1989, and in February and April 1990. Trapsite D was located two miles southeast of Stephenville in Erath County, Texas. This site was trapped from October through December 1989, and February, June, and July of 1990. Trapsite E was located one mile east of Stephenville, Texas, in Erath county. Traps were set from February through April, 1990, and again in June and July, 1990. Trapsite F was located 2 miles south of Hico, Texas, in Hamilton County. Traps were set during February and April of 1990.

Two types of live traps were used: Sherman live traps and a custom made, treadle variety. The Sherman live traps were 5.08 cm wide, 6.35 cm tall, and 16.51 cm long. The custom made traps were 5.08 cm wide, 6.35 cm tall, and 30.48 cm long. Bait consisted of either birdseed, oatmeal, sunflower fruits, dog food, or a combination of any of these. Collected specimens were given identification numbers that corresponded to the trapsite and held in individual cages with food and water until testing. All small mammals were identified to species. Specimen data also included date of capture, sex, life stage, and number of ticks present. Life stage was determined by fur texture and molt patterns.

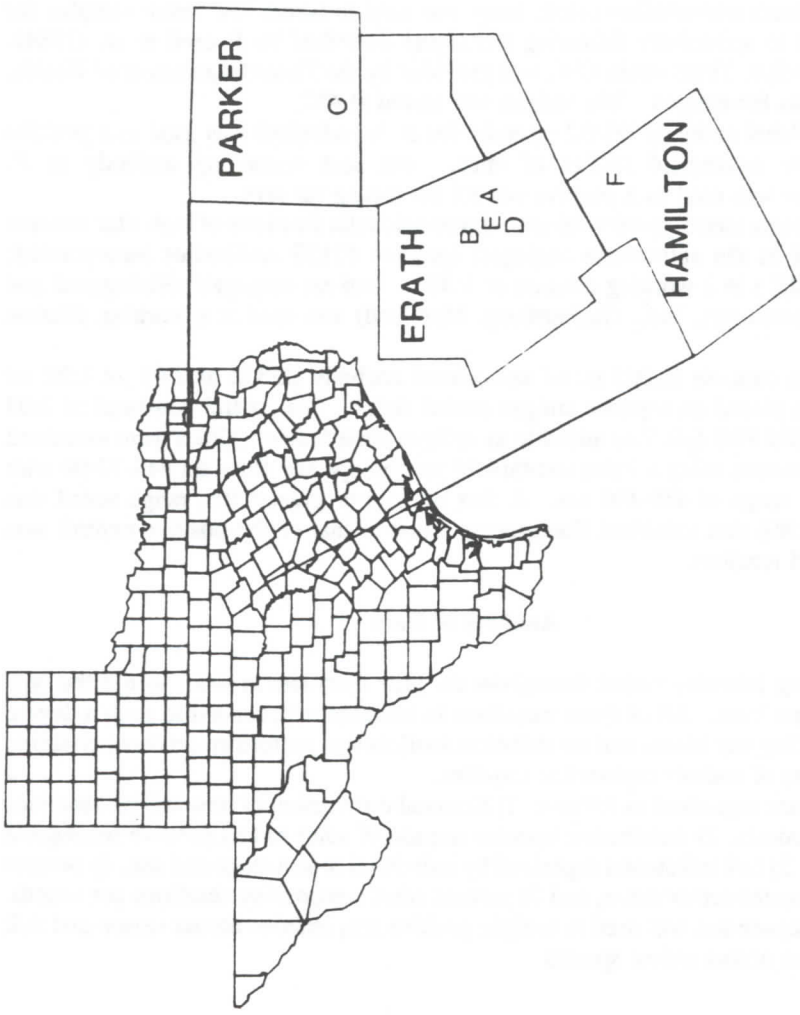

Figure 1. Trapsite locations in North Central Texas. One trapsite was located in each of Parker and Hamilton Counties, while four trapsites were in Erath County. All are designated with a capital letter in the approximate location within the county.

Preparation of Serum Samples

All dissections were conducted under a laminar flow hood with ultraviolet lights to minimize contamination of tissues. Dissection instruments were flame sterilized. Each specimen was killed by cervical dislocation and immediately bled. The blood was placed in a 1.5 ml microfuge tube, allowed to sit for 20 minutes to ensure clotting, and centrifuged at 2000 rpm (325 X G) for 10 minutes. The serum was aspirated using a sterilized capillary pipet and placed in another labeled microfuge tube. The tubes of sera were stored at -20°C.

Serological Studies

An indirect immunofluorescent assay was used to screen the serum samples for antibodies to spirochetes following techniques described by Russell at al. (1984). *B. burgdorferi*, Texas strain GW, was provided by the Texas Department of Health, and used as the antigen. The antigen was stored at 4°C.

Monoclonal antibody H5332 (specific for *B. burgdorferi*) was used as a positive control for serological studies of mice. Rat sera containing antibody to *B. burgdorferi* was used as a positive control for testing rat sera.

Fluorescein isothiocyanate labeled immunoglobulin fractions of high titer antisera were used as the anti-mouse conjugate (product #2150 Antibodies Incorporated; Davis, Calif.) in a working dilution of 1:400. Anti-rat conjugate (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Maryland) was used at a working dilution of 1:40.

Positive controls (0.003 ml of monoclonal antibody H5332 or 0.03 ml 1:32 rat sera) were placed on separate antigen coated slides. The control consisted of 0.03 ml of 0.03M PBS (pH 7.6) added to an antigen coated slide. Slides were examined for fluorescence using a Zeiss standard 16 microscope and filter set #48-77-09 with excitation range of 450-490 nm. A titer of 1:20 or greater (the range tested was 1:20 to 1:80) that exhibited fluorescence equal to that of the positive control was considered reactive.

Analysis of Data

Trapping intensity varied throughout the year from site to site, as did the type of traps and baits. All of these variations in techniques fluctuated to such a degree that sampling was biased and no statistical analysis was performed on data pertaining to numbers of rodents captured at trapsites.

Data were organized as follows: 1) Seasonal distribution of seropositive reactions in adult rodents, 2) distribution between trapsite of adult rodent positive serological reactions, 3) tick infestation organized by individual rodent stage and sex, 4) percent of each species seropositive, and 5) percent adult seropositive reactions per month. The Chi-square test was used to analyze positive sera reaction across season and tick distribution across rodent species.

RESULTS

Several species of mammals not listed in previous literature were shown to exhibit

positive serological reactions. These species include *Baiomys taylori* (Thomas), *Peromyscus attwateri* (J. A. Allen), *Reithrodontomys fulvescens* (Allen), and *Reithrodontomys montanus* (Baird). At the time this study was conducted, *Sigmodon hispidus* (Say and Ord) had not been reported to exhibit a positive reaction, but Oliver et al. (1995) have recently characterized Lyme disease in this rodent species.

The seropositive rodents were captured in three different counties: Erath (10 positive), Parker (four positive), and Hamilton (four positive). Although *P. leucopus* had the greatest number of seropositive specimens, it did not have the highest percent of positive serological reactions. *R. montanus* demonstrated the highest percentage of positive serological reactions, but only six representatives of this species were collected and only one was seropositive.

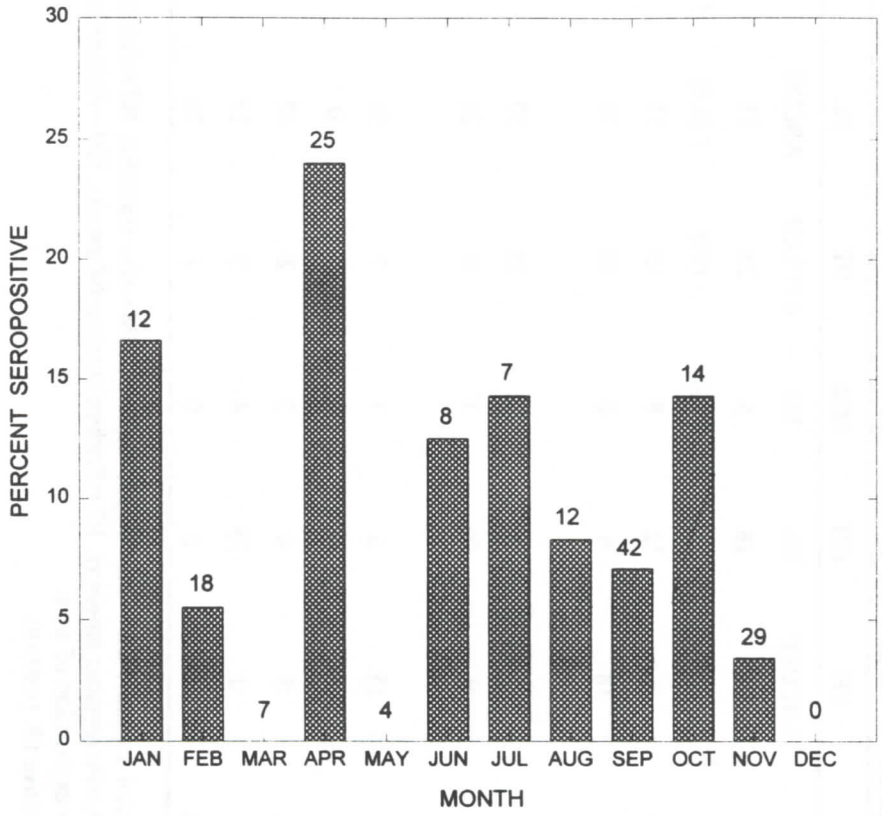

Figure 2. Adult sample size and percent seropositive rodents per month. Numerals above bars indicate sample size; bar heights represent the percent of seropositive specimens collected during the month. The data are grouped for all species of rodents tested during the month.

Table 1. Summary of trapping and biological data collected during study for each species of rodent tested.

Species†	SH	CH	MM	BT	PL	PA	RF	RM
Trap site‡	ABCDEF	BA	BD	ABCDEF	ABCDE	CA	ACEF	DF
# captured	24	18	8	25	81	24	28	6
# positive§	1 (4.2)	0 (0)	0 (0)	3 (12)	7 (8.6)	3 (12.5)	3 (10.7)	1 (16.6)
male	6	12	8	15	51	14	12	3
female	18	6	0	10	30	10	16	3
Stage								
adult	24	16	5	25	57	18	27	6
juvenile	0	2	3	0	24	6	1	0
Season								
autumn	15	3	1	7	31	2	7	2
winter	4	0	0	5	9	10	8	4
spring	5	0	3	8	22	6	13	0
summer	0	15	4	5	19	6	0	0
# with ticks	2	0	0	1	16	2	1	0

†SH=*Sigmodon hispidus*, CH=*Chaetodipus hispidus*, MM=*Mus musculus*, BT=*Baiomys taylori*, PL=*Peromyscus leucopus*, PA=*Peromyscus attwateri*, RF=*Reithrodontomys fulvescens*, RM=*Reithrodontomys montanus*.
‡See text for explanation of sites.
§Number positive (% positive).

The month of April had a larger percent (24%) of seropositive rodents than the other months of the year, which corresponds with adult population peaks in several rodents (Fig. 2). This is also an active time for nymphal and larval ticks of certain species to be foraging. During April, species that were seropositive included *P. leucopus* (one), *P. attwateri* (one), *R. fulvescens* (three), and *B taylori* (one). April was the only month during which more than one species was seropositive. There was no significant difference among seropositive specimens between seasons ($X^2=2.45$, df=3, $P>0.05$).

None of the 36 juvenile rodents in this study was seropositive. Fifteen of the 121 male rodents captured (12.3%) were seropositive, while 3 of 93 (3.2%) of the females were seropositive.

All ticks collected during the study were in nymphal or larval stages. Three species were identified - *Dermacentor variabilis* (Say), *Amblyomma americanum* (L.) and *Ixodes scapularis* Say. *D. variabilis*, *A. americanum*, and members of the genus *Ixodes* harbor *B. burgdorferi* (Anderson et al. 1983, 1985, 1986; Piesman and Sinsky, 1988; Rawlings et al., 1987; Anderson and Magnarelli, 1980).

Tick infestation was significantly different across rodent species ($X^2=14.176$, df=7, $P<0.05$), with the value for *P. leucopus* accounting for over half of the Chi-square value of 14.176. Species without ticks had values that only accounted for about one-fifth of this value. This suggests that percent tick infestation is biased by *P. leucopus*, and that there may be very little host preference in other rodent species. Of 36 juvenile rodents collected, 4 (11.1%) were infested with ticks. A total of 18 ticks were found on 178 adult rodents, a 10.1% infestation. When sorted by sex, 10 of 121 male rodents (8.3%) and 12 of 93 female rodents (12.9%) were infested with ticks.

DISCUSSION

Previous research from the three geographic foci of Lyme borreliosis implicated *P. leucopus* as a major reservoir of the disease. The percent of adult rodents that exhibited a serological reaction in this study (10.1%) is similar to the serological reactions of adult rodents in the midwest (6%) (Anderson et al., 1987b), although not as high as the percentages along the Atlantic Coast (17-50%) (Magnarelli et al., 1984b, 1984c).

Habits of the two families of rodents trapped during the study period were researched to determine chance of exposure to the tick transmitted spirochetes. Several factors, including habitat, activity, home range, and tick infestation seem to play roles in the possibility of exposure. The two species that had no specimens exhibiting a serological reaction were *Chaetodipus hispidus* (Baird) and *Mus musculus L.* Both species frequent areas not conducive to tick infestation. *C. hispidus* prefers dry habitats, where foraging ticks are less likely to be found. According to Dalquest and Horner (1984), parasites are almost never found on this species. Thomas et al. (1990) reported eight species of ectoparasites on *C. hispidus*, but none of them were ticks. *M. musculus*, with its close association to human habitations, could be exposed to areas that have been treated with acaricides, or other pest control. Although both species can be active year round, *C. hispidus* can also become inactive in winter and *M. musculus* has been known to inhabit man-made structures during that time. The home range of both of these species is

particularly small. *C. hispidus* has a home range of 0.30 ha (Schmidly 1983) and *M. musculus* has a home range of only 0.23 ha (Waggoner, 1974). Schmidly (1983) also noted the sedentary behavior of these two species, which would decrease the likelihood of contact with an infected tick vector.

No ticks were found on *R. montanus*. This species prefers well drained, climax grasslands or blackland prairies, typically avoiding tall grass. These habitats are not ideal for foraging ticks. Dalquest and Horner (1984) noted that ectoparasites were seldom found on this species. The small sample trapped in this study did not have ectoparasites, but one specimen exhibited a positive serological reaction indicating possible contact with an infected vector. A larger sample size would have provided better data to test the relationship between *R. montanus* and possible tick vectors. All other species trapped use habitats conducive to tick infestation (tall grass, moist vegetation) and were active year round. Home ranges varied, but all were bigger than *C. hispidus* and *M. musculus*.

With the exception of *R. montanus*, species that exhibited a positive serological reaction also had specimens with tick infestation. Tick infested rodents harbored both larval and nymphal stages. In the case of Lyme disease, an infected mouse can pass *B. burgdorferi* on to the tick vector, which typically maintains the disease transtadially (Spielman et al., 1984). After molting, the adult tick vector can then feed on larger mammals, including humans.

Timing of exposure to spirochetes can affect the results of serological tests. *P. leucopus* exhibits an early IgM response followed by an IgG response (Schwan et al. 1989). The initial IgM response in *P. leucopus* can be detected as early as one to two days after infection. Schwan et al. (1989) demonstrated that the IgG1 and IgG2 response in *P. leucopus* increased during the 84 days of their experiment. Also, antibody response may differ with the rodent species tested.

Fifteen of the 18 positive serum reactions in the 214 specimens examined were male. This might indicate a higher susceptibility among males, or perhaps a prolonged antibody response. There are differences in the activity between males and females in several of the species assayed (Schmidly, 1983). If the males of the species were more active, they would be more likely to come in contact with foraging ticks, thus they would be more likely to become infected. In the current study, however, roughly equal percentages of males (10/122=8.9%) and females (12/93=12.9%) were infested with ticks.

The lack of seropositive reactions in juveniles may be attributable to their immunological incompetence. Golub (1977) determined that mice do not generate an antibody response until at least one week of age. Antibody response is not equal to that of an adult mouse until at least 3 weeks of age. Moody et al. (1990) experimentally infected rats with *B. burgdorferi* at one week of age. Two weeks after inoculation, antibodies were detected using an ELISA. Shih and Pollack (1992) determined that *B. burgdorferi* had to multiply locally in the skin for several days before becoming systemic, which might further complicate the antibody response in a young mouse.

The Chi-square detected randomness of positive serum reaction across season. Along the Atlantic Coast there is typically a peak of infection in rodents in the spring. Results of this study indicate that statistically there is no such peak in North Central Texas, possibly due to the warmer winters in Texas that allow ticks to remain active year round. Three species (*P. leucopus*, *P. attwateri*, and *B. taylori*) were trapped year round, and each had seropositive specimens. Year round activity

could play a major role in spirochete maintenance, thus increasing the likelihood of susceptibility.

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