The Prevalence of *Borrelia burgdorferi*, the Lyme Disease Spirochete, in Ticks and Rodents in Northeast Arkansas

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Abstract

Lyme disease, caused by the spirochete, *Borrelia burgdorferi*, has been reported from 36 of Arkansas' 75 counties. Ticks and wild rodents from nine northeast Arkansas counties were surveyed to determine the prevalence of *Borrelia* infection in potential tick vectors and reservoir host populations. Indirect immunofluorescent assays with murine monoclonal antibody H5332, specific for *B. burgdorferi*, detected a 2.1% rate of infection for the 638 ticks surveyed and an 11.8% infectivity rate for the 102 rodents surveyed.

Introduction

Lyme disease is a tick-borne bacterial infection that was initially identified in the mid-1970's as a form of juvenile arthritis (Steere et al., 1977). The etiologic agent is a spirochete, *Borrelia burgdorferi*, first isolated in 1982 by Burgdorfer et al. (1982). Humans most often acquire the disease when bitten by an infected vector.

The incidence of Lyme disease is difficult to estimate because of variability among states in reporting requirements, case definitions, and surveilance methods (Cielsielski et al., 1988). Nevertheless, Lyme disease is considered to be the most commonly reported vector-borne disease in the United States (Miller et al., 1990). In Arkansas 99 cases of Lyme disease have been reported from 1988 to 1992 (T. McChesney, 1993, pers. comm.).

Epidemiological evidence suggests that the Lyme spirochete is maintained in the wildlife population by reservoir hosts and infected overwintering vectors. Through either bacterial isolation or serological surveys, this organism has been associated with a wide variety of wild and domestic animals including white-footed mice, chipmunks, raccoons, skunks, squirrels, ground-feeding birds, rabbits, white-tailed deer, horses, cows, dogs, and cats (Anderson et al., 1983; Anderson and Magnarelli, 1984; Anderson et al., 1985).

The pattern of spirochete transmission appears to involve larval or nymphal ticks acquiring the spirochete by feeding on an infected reservoir host. This infection is carried through to either the nymph or adult stage (transstadial passage) which, while feeding, infects the next host. Humans typically acquire the infection when fed upon by infected nymphs.

The transmission cycle is most clearly understood in the Northeast because of the concentration of research conducted there. In this endemic Lyme-disease region, *Ixodes dammini*, the deer tick, is the principal vector implicated in the cycle and *Peromyscus leucopus*, the white-footed mouse, is considered the major reservoir host (Burgdorfer et al., 1982; Burgdorfer, 1984; Anderson et al., 1985; Spielman et al., 1985). The infectivity rate for these mice has been as high as 86% in endemic foci (Anderson et al., 1985). A natural infection rate of 35% has been reported for the deer tick in this area (Anderson et al., 1983; Magnarelli and Anderson, 1988).

Although the epidemiological picture for Lyme diseae is well-constructed in the Northeast, little is known about its status in the South where there is an increasing incidence (Burgdorfer and Gage, 1987; Cielsielski et al., 1988). Several investigations into possible wildlife reservoirs and potential tick vectors have helped to initiate a better understanding of Lyme disease ecology in the southern states. Burgdorfer and Gage (1987) determined that the hispid cotton rat, *Sigmodon hispidus*, is capable of developing a *Borrelia burgdorferi* infection and, when spirochetemic, can be infective for the nymphs of feeding *Ixodes scapularis*, the black-legged tick. They further observed that this tick is also capable of transmitting the spirochete. Two other species of ticks, *Amblyomma americanum*, the lone star tick, and *Dermacentor variabilis*, the American dog tick, have been found to be naturally infected (Schulze et al., 1984; Anderson et al., 1987; Magnarelli and Anderson, 1988; Feir and Reppel, 1990). All three of these tick species are found in abundance in Arkansas.

The purpose of this study was to address three main questions relevant to the epidemiology of Lyme disease in northeast Arkansas: (1) What tick(s) potentially serve(s) as vector(s) for *B. burgdorferi*?; (2) What is the prevalence of *B. burgdorferi* in the potential tick vector population(s)?; and (3) What is the prevalence of *B. burgdorferi* infection in potential wild rodent reservoirs? Such information should help in the construction of an epidemiological picture for Lyme disease in this area. Identification of the most commonly encountered vector(s) could be useful in defining seasonal and locational

risk factors. Estimation of the prevalence of *Borrelia* infection in both potential reservoir hosts and arthropod vectors is necessary to accurately asses the threat that Lyme disease poses to people living within the study area.

Material and Methods

Collection and Examination of Ticks.--The ticks examined in this study were collected from several locations in Clay, Craighead, Fulton, Greene, Izard, Lawrence, Poinsett, Randolph, and Sharp counties in northeast Arkansas. Specimens were obtained either by removal from their mammalian hosts within a study site or by flagging vegetation in the various study areas. Collected ticks were placed in bags containing a moist paper towel until they could be examined in the laboratory. Ticks were identified to species, sex, and developmental stage.

After identification, the internal body content of each tick was dissected and smeared in a drop of phosphate-buffered saline on a ten-well, teflon-coated glass slide. This film was examined by darkfield microscopy. Those slides on which spirochetes were detected were air-dried, fixed in methanol, and examined by indirect immunofluorescence. Murine monoclonal antibody (mAb) H5332, prepared against *Borrelia burgdorferi*, (compliments of Dr. Alan G. Barbour, University of Texas Health Sciences Center, San Antonio, TX) and fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin G (IgG) were used in the indirect fluorescent-antibody (IFA) staining (Anderson and Magnarelli, 1984).

Isolation and Cultivation of Borrelia *from Feral Rodents.*--Rodents were collected either by Museum Special snap traps or by Sherman box traps (H. B. Sherman Co., Deland, FL) from Clay, Craighead, Fulton, Greene, Lawrence, Poinsett, Randolph, and Sharp counties in northeast Arkansas. Those animals captured in box traps were euthanized with chloroform. All specimens were identified as to species and prepared for necropsy by removal of abdominal hair with a depilatory. After hair removal, the animals were placed in a 5.25% sodium hypochlorite solution for three minutes, rinsed with sterile water, and painted with an iodine-alchol mixture to minimize microbial contamination.

BSK-II medium (Barbour, 1984; Berger et al., 1985; Barbour, 1986) was used for bacterial cultivation. After preparation, the medium was filter sterilized through a 0.22 micron filter and dispensed into sterile, screw-up culture tubes. After the animals were prepared for necropsy, the spleen and urinary bladder were aseptically removed and triturated in 2 ml of BSK-II medium with a sterile

Dounce homogenizer. A 1 ml portion of this extract was inoculated into a sterile culture tube containing BSK-II medium. The tubes were incubated at 34°C for 6-8 weeks. Based on previous work (Anderson et al., 1985; Anderson et al., 1987; Schwann et al., 1988), each culture was examined weekly for spirochetes by darkfield microcopy for the first 3 weeks and periodically thereafter.

Results

Tick Data.--A total of 638 ticks from a nine-county area (Fig. 1) was collected from fall 1989 through fall 1991 and examined for *Borrelia burgdorferi*. Four different species were represented in this total as were both sexes and two life cycle stages (Table 1). Of the ticks collected and examined, 13 (2.08%) contained spirochetes which reacted positively with murine mAb H5332 specific for *Borrelia burgdorferi* (Table 1). *Amblyomma americanum* and *Ixodes scapularis* were the two tick species most commonly encountered in both the general and the *Borrelia*-infected tick population (Table 1). Geographically, tick specimens exhibiting a positive antibody reaction were found in only four of the nine counties surveyed (Fig. 1).
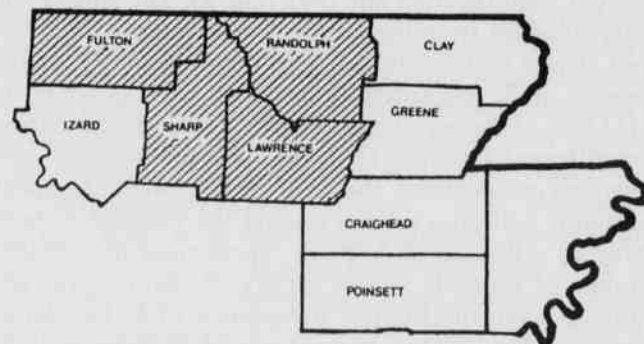

Fig. 1. Northeast Arkansas counties included in the tick survey. (Counties in which infected ticks were collected are cross-hatched).

Table 1. Comparison of the general tick population with the *Borrelia*-infected tick population for ticks collected from northeast Arkansas from fall 1989 throught fall 1991.

Species	Sex	Stage	Total Infected	Total
Amblyomma americanum (lone star tick)	5/132 f	2/55 n	7	200
	2/68 m	5/145 a		
Dermacentro variabilis (American dog tick)	0/101 f	0/6 n	1	180
	1/79 m	1/174 a		
Ixodes scapularis (black-legged tick)	0/126 f	0/0 n	5	225
	5/99 m	5/225 a		
Rhipcephalus sanguineus (brown dog tick)	0/15 f	0/17 n	0	21
	0/6 m	0/4 a		
	5/374 f	2/78 n	13	626
	8/252 m	11/548 a		

KEY: f = female; m = male; n = nymph; a = adult

Rodent Data.--A total of 102 rodent specimens, representing 10 species, was collected in an eight-county area from fall 1989 through fall 1991 (Fig. 2). The spleen and urinary bladder from each animal were removed and cultured. These tissue cultures were then examined for the presence of *B. burgdorferi*. Spirochetes were observed in 12 (11.8%) of these cultures. These 12 animals came from four different counties (Fig. 2). *Sigmodon hispidus* and *Peromyscus maniculatus*, the deer mouse, were the most-commonly collected species among the infected rodents (Table 2). Although *S. hispidus* represented the greatest number of animals in the survey, *P. maniculatus* was found to have the highest prevalence of *B. burgdorferi* infection. The overall prevalence of *Borrelia*-infected rodents from Randolph, Craighead, Greene, and Poinsett counties was 18.8%, 16%, 14.3%, and 5.5% respectively.
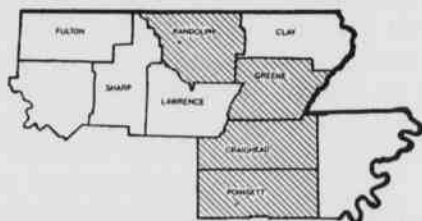

Fig. 2. Northeast Arkansas counties included in the rodent survey. (Counties in which infected rodents were collected are cross-hatched.)

Table 2. Rodent species and numbers collected from northeast Arkansas from fall 1989 through fall 1991 and examined for *B. burgdorferi* infection.

Species	No. infected	No. total	%
Microtus pinetorum (Woodland vole)	0	7	0.0
Mus musculus (House mouse)	0	19	0.0
Neotoma floridana (Eastern wood rat)	0	1	0.0
Oryzomys palustris (Marsh rice rate)	1	5	20.0
Peromyscus gossypinus (Cotton mouse)	1	9	11.1
Peromyscus leucopus (White-footed mouse)	1	9	11.1
Peromyscus maniculatus (Deer mouse)	5	15	33.3
Reithrodontomys fulvescens (Fulvous harvest mouse)	0	2	0.0
Sigmodon hispidus (Hispid cotton rat)	4	33	12.1
Tamias striatus (Eastern chipmunk)	0	1	0.0
unknown	0	1	0.0
TOTAL	12	102	11.8

Discussion

Since 1988, 36 of Arkansas' 75 counties have reported a total of 99 cases of Lyme disease. Of the nine counties included in this survey, five (Craighead, Greene, Poinsett, Sharp, Randolph) have reported at least one case of Lyme disease within the last six years. In this study, either tick midgut smears or rodent tissue cultures which reacted positively with mAb H5332 were detected from all five counties in which Lyme disease has been reported (Figs. 1 and 2). Additionally, three positive ticks were found in two counties (Fulton and Lawrence) where no human cases of Lyme disease have yet been reported. These findings help substantiate the clinical diagnosis of Lyme disease since they provided evidence of the presence of both the etiologic agent and infected tick vectors.

Ixodes scapularis has been proposed as the potential major vector in the southeast U.S. because of its relationship to the major vector in the Northeast, *I. dammini*, and its proven vectorial capacity in the laboratory (Burgdorfer and Gage, 1987; Piesman and Sinsky, 1988). Natural infection rates of *I. scapularis* have been quite low (<1%) as compared to the natural infection rates for *I. dammini*

which have been as high as 35% (Anderson et al., 1983; Magnarelli et al., 1986; Magnarelli and Anderson, 1988; Piesman and Sinsky, 1988). Recent evidence, however, suggests that *I. scapularis* and *I. dammini* are not distinct species and should not be separated as such (Oliver et al., 1993). If this is true, then the importance of *I. scapularis* cannot be understated despite the differences in the reported prevalence of *Borrelia* infection. It is possible that the variation in the natural infection rates between these two ticks is due more to reservoir host infection rates than to differences in vectorial capacity.

In this study, 2.2% of the *I. scapularis* were determined to react positively upon IFA analysis. All of these ticks were adults and were obtained form white-tailed deer. Since all the positive black-legged ticks in this investigation were attached to an animal host at collection, it was impossible to determine if the ticks were infected prior to feeding or if the ticks ingested the bacteria while feeding on a spirochetemic host. The former case suggests acquisition of the infection as a nymph with subsequent transstadial passage and implies a potential ability of the tick to transmit the organism to another host. The latter scenario neither supports nor refutes vectorial capacity.

Evidence gathered in this study also suggests another important vector for Lyme disease in northeast Arkansas, *Amblyomma americanum*. The lone star tick showed the greatest infectivity rate, 3.5%, of the ticks examined. Feir and Reppell (1990) reported a similar infection rate (2.3%) for this tick in Missouri. Other populations of naturally-infected *A. americanum* in Alabama, North Carolina, and New Jersey have also been found (Schulze et al., 1984; Magnarelli et al., 1966; 1991). However, the efficiency of *Amblyomma* as a vector for *Borrelia burgdorferi* has been questioned. Piesman and Sinsky (1988) reported that the capacity of the lone star tick to acquire *Borrelia* infection varied among populations. They further noted that if infection was established, the organisms were lost in transstadial passage.

Conversely, the lone star tick has been strongly associated with the development of an erythema migrans rash in a number of Lyme disease cases diagnosed at a family clinic in Cape Griardeau, Missouri (Masters, 1990). In every instance immunological testing confirmed the diagnosis of Lyme disease. Outside of the southern U.S., *Amblyomma* has been implicated as a potential vector in New Jersey (Schulze et al., 1984).

In this investigation both nymphs and adults of *A. americanum* were found to be infected with *Borrelia* (Table 1) and the majority of these were collected from flagging vegetation. Similarly, Schulze et al. (1984) also reported spirochetes from both nymphs (22%) and adults (5.8%) of this species. A major question raised by the positive ticks swept from vegetation concerns the time their

infection was acquired. One possibility is that the ticks were infected with the spirochete as larvae or nymphs and the infection was passed transstadially into the respective nymphs or adults. Piesman and Sinsky (1988) reported that transstadial passage of *Borrelia burgdorferi* in *A. americanum* was non-existent in the metamorphosis from larva to nymph. Published information was not found documenting the success of transstadial passage from nymph to adult in the lone star tick. It is feasible that, even if the spirochete can not be transstadially passed from larva to nymph, a passage from nymph to adult is possible. Another likely explanation for infection within a given stage is that the infection may have been acquired in that stage, and the ticks simply have not yet molted. In the first situation, an infected tick could serve as a vector for a new host. In the latter scenario, the vectorial capacity of a tick lies in its ability to transstadially pass the spirochete since the tick has already fed.

In addition to the lack of evidence supporting transstadial passage of *B. burgdorferi* in *Amblyomma*, there have been no published reports of the successful transmission of *Borrelia burgdorferi* to a host by infected *A. americanum* ticks within the confines of a laboratory setting. Until these relationships can be conclusively established, the vectorial capacity of *A. americanum* for the Lyme disease spirochete can only be hypothesized.

One other potential tick vector in northeast Arkansas is *Dermacentor variabilis*, the American dog tick. Examination of 180 *D. variabilis* ticks yielded one (0.55%) positive adult male. Naturally-occuring infection rates of 1.3% have been reported for the American dog tick in Missouri (Feir and Reppell, 1990). Other populations of *Borrelia*-infected nymphs and larvae have been reported from the eastern U.S. (Anderson et al., 1985; Anderson et al., 1987; Magnarelli and Anderson, 1988). However, at this time there is no convincing published evidence linking the bite of *D. variabilis* to the development of an EM rash in humans nor have any known laboratory experiments demonstrated the American dog tick to transmit the Lyme spirochete to a specific host.

This investigation does not address the actual effectiveness of any given tick species as a vector of Borrelia burgdorferi to humans. Rather, this study was designed to clarify which tick(s) could serve as Borrelia vectors in northeast Arkansas and to determine the extent of infection in the tick population. The data gathered suggest that *Amblyomma americanum* and *Ixodes scapularis* are the most likely candidates for Lyme vectors.

Another key aspect of the Lyme disease epidemiological picture in northeast Arkansas is the maintenance of the spirochete in reservoir host populations. These hosts serve as overwintering agents for *B. burgdorferi* and as reservoirs of infection for feeding tick vectors (Anderson et al., 1987). Isolation of spirochetes from feral rodents

has been suggested as a method for identifying endemic areas of Lyme disease (Anderson et al., 1985). Burgdorfer and Gage (1987) determined that the hispid cotton rat, *Sigmodon hispidus,* was a competent reservoir for the spirochete and suggested field studies of wild rodents to further elucidate the ecology of *Borrelia burgdorferi* in the South.

In the present investigation, an 11.8% infectivity rate was noted among the 102 rodents examined. The Lyme disease organism was cultured from the following five species of the 10 different species studied: *Oryzomys palustris* (the marsh rice rat), 20%; *Peromyscus gossypinus* (the cotton mouse), 11.1%; *Peromyscus leucopus* (the white-footed mouse), 11.1%; *Peromyscus maniculatus* (the deer mouse), 33.3%; and *Sigmodon hispidus* (the cotton rat), 12.1%. These infection rates correlate with results from a similar survey conducted across North Carolina, South Carolina, Georgia, Florida, Alabama, and Mississippi by Magnarelli et al. (1992). These investigators found infection rates for *P. gossypinus* of 21.7%, 37.9%, 35.0%, 15%, and 17.3%, respectively, for the aforementioned states. Additionally, 30% of *P. leucopus* examined from North Carolina possessed antibodies to *B. burgdorferi.*

All of the *Borrelia*-positive rodents were identified from counties where human cases of Lyme disease have been reported. The relative importance of any particular rodent species as a *Borrelia* reservoir goes beyond the scope of this project. It is evident that *Borrelia burgdorferi,* or a closely-related spirochete, exists in both potential tick vectors and reservoir host populations in northeast Arkansas. Further investigations should include an intensive study of identified tick vectors followed by feral rodent surveys in those areas where positive vectors have been recovered.

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