PROCEEDINGS OF THE 2ND WORKSHOP ON LYME DISEASE IN THE SOUTHEAST

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Sept. 7, 1993

Lyme Disease Epidemiology and Tick Ecology

Presiding: James H. Oliver, Jr.

Lyme Disease Surveillance, Ticks Vectors, & Tick Hosts in the Southeast

Presiding: Durland Fish

Sept. 8, 1993

Isolation & Transmission of *Borrelia burgdorferi*

***Presiding*:** Gary R. Mullen

Future Research on Lyme Disease in the Southeast

***Presiding*:** Daniel E. Sonenshine & Charles S. Apperson

BREAK-OUT GROUP REPORTS

Sept. 9, 1993

Tick Systematics and Speciation

***Presiding*:** Joseph F. Piesman

INTRODUCTION

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These proceedings contain presentations made at the second workshop on Lyme disease in the southeastern United States. Lyme disease case information and results of preliminary field studies were reported at the first workshop, held in Knoxville, Kentucky on July 29-31, 1991. Since this workshop, reported cases of Lyme disease in the southeastern U. S. have continued to increase southward beyond endemic areas in the northeast. Information on the epidemiology and epizootiology of Lyme disease has been accrued mainly from research conducted in endemic areas of the northeast, midwest and northwest. Knowledge of the reservoir hosts and tick vectors involved in the transmission of *B. burgdorferi* in the southeast is comparatively lacking. However, a considerable amount of new information and, numerous applied and basic research studies on Lyme disease in the southeast have been conducted during the past two years. Accordingly, a second Lyme disease workshop was held with the following objectives:

(1) to share information on recent research findings;

(2) to provide a forum for the exchange of ideas;

(3) to promote awareness of Lyme disease, especially in the southeastern U. S.; and

(4) to target areas for future research efforts.

LYME DISEASE TRANSMISSION RISK: NORTH AND SOUTH

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This report summarizes personal experiences with the *Ixodes* vectors of Lyme disease in the eastern United States. Beginning in 1975, I have studied the ecology of *Ixodes* ticks with an eye toward understanding the transmission dynamics of several human pathogens, including *Borrelia burgdorferi* and *Babesia microti*. Study areas where I have had the opportunity to observe these ticks include sites in Maine, Massachusetts, New York, New Jersey, Maryland, Virginia, Georgia, and Alabama. Thanks are extended to the "hosts" for these study areas including Drs. R. Smith, A. Spielman, D. Fish, T. Schulze, D. Sonenshine, and J. Oliver.

Similarities and differences exist in the northern and southern populations of the deer tick, labeled "*I. dammini*" and "*I. scapularis*", respectively. Similarities exist mainly in the habits of the adult ticks. They are found in abundance during the cooler months on deer from Maine to Florida. Although their distribution is spotty, they are found in approximately equal abundance on deer where they are well established. Collecting adults by flagging or dragging is a relatively easy task. The habits of the immature ticks vary markedly from the north to the south. The primary hosts for northern populations of the deer tick are rodents. I have found >100 immature *Ixodes* on a white-footed mouse in Massachusetts. In contrast, southern populations rarely infest rodents. The average number of immature *Ixodes* on rodents in the southern U. S. is usually <1. In addition, the questing habits of the nymphal stage differs dramatically. In Massachusetts, New York, and New Jersey, >30 nymphs can be collected in 1 hr. Recently, heroic attempts to flag nymphal *I. scapularis* on islands off the coast of Georgia resulted in the

collection of 10 nymphs in 12.5 h of collecting. A comparable effort on Nantucket Is. would have resulted in the collection of ca. 375 nymphs. I believe this deficit is due to the different host range and questing habits of the 2 respective northern and southern populations of ticks. The reduced infection rate in southern nymphal ticks also results in a reduced transmission risk to humans. The infection rate of northern ticks with *B. burgdorferi* is generally ca. 25% in nymphs. Spirochetes are rare or absent in southern nymphal *Ixodes*. In summary, human risk of acquiring Lyme disease differs dramatically from north to south in the eastern U. S. A key point of interest may be the "transition zone" along the Maryland-Virginia border.

LYMSIM, A MODEL OF *IXODES SCAPULARIS* POPULATION DYNAMICS AND TRANSMISSION OF THE LYME DISEASE AGENT, *BORRELIA BURGDORFERI*

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A model, LYMSIM, simulates the population dynamics and management of the Lyme disease vector, *Ixodes scapularis*. The model also simulates transmission of the Lyme disease agent, *Borrelia burgdorferi*. LYMSIM provides a tool to study the effects of weather, habitat, and density and type of host animal on tick population growth. Population densities, seasonal activity and growth rates over a wide range of geographic areas and weather patterns are used as an indicator of model acceptability during calibration of biological parameters.

LYMSIM is validated by comparison of simulated and observed tick population data and *B. burgdorferi* infection rates. Further validity is indicated by comparisons of simulated and reported seasonal activity patterns at various locations in the United States.

The model is used to study relationships between density of white-tailed deer and tick population growth. Furthermore, the model is used to study the relationship between a key reservoir host, the white-footed mouse, and level of infection in tick vector and animal host populations. LYMSIM includes effects of control technologies on tick populations and can be used to develop strategies for management of *I. scapularis*.

The model software is written in Microsoft Professional Basic for interactive operation on IBM or compatible microcomputers.

GIS AND LYME DISEASE

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Assessing the risk of vector-borne diseases, in general, and Lyme disease, specifically, is difficult because of the need to extrapolate patterns observed locally to broad, geographic regions. This problem is primarily due to the intensive nature of collecting field data which restricts the areas that can be sampled.

Because arthropod vectors are influenced by local environmental conditions, we have investigated the use of mappable environmental variables as surrogate measures of disease risk and vector abundance. Geographic information systems (GIS) are protocols to input, store, retrieve, manipulate, analyze and output

spatial data. As computerized procedures, they provide a means of mapping disease risk and vector abundance over large areas (thousands of square kilometers) at high resolution.

In this presentation, we discuss two case studies that link GIS with disease and vector studies. The first combines GIS with standard epidemiological analyses to assess human disease risk for Lyme disease. The second combines GIS with a vector survey of *Ixodes scapularis* removed from white-tailed deer to predict vector abundance.

A case-control study was conducted using GIS to identify environmental risk factors for Lyme disease in Baltimore County, Maryland. Baltimore County, which covers 165,000 ha, has had the largest numbers of incident cases in Maryland since 1989. GIS was used to locate the residences of cases of Lyme disease in 1989 and 1990. As controls, approximately 500 randomly selected residences were obtained. A total of 58 environmental variables were assessed at the locations of cases and controls by retrieving information from maps of the county with approximately 100 m resolution. The extracted information was analyzed by traditional case-control methods. GIS was used to generate a map of Lyme disease risk from the resulting epidemiological model.

To test the model, the distributions of cases in 1991 and a new group of random controls were compared relative to the risk map. Cases were at least 16 times more likely to reside in high risk areas than in low risk areas. As a second test, the habitat around capture sites of infected white-footed mice were consistent with the risk factors identified for human disease.

In the second study, adult *I. scapularis* were collected from hunter-killed deer in Kent County, Maryland. The goal of the study was to identify environmental factors that could predict vector abundance on this host species. Voronoi tessellation was used to identify sampling sites for deer and the environmental characteristics within the polygons were measured for 42 variables. Multiple linear regression was used to try to predict adult tick abundance based on the environmental characteristics of the polygons. There was a significant association between vector abundance and several environmental factors. In more than two-thirds of the polygons the difference between the observed and predicted numbers of ticks was two or less.

These examples suggest that combining GIS and various traditional field techniques may allow us to predict disease risk, or potential risk, over large areas at high resolution with a high degree of accuracy.

LANDSCAPE ECOLOGY OF LYME DISEASE IN WESTCHESTER COUNTY, NEW YORK

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Westchester Co., NY is a suburban residential community that forms the northern border of New York City. This single county annually reports nearly 20% (1500-1800) of the nation's Lyme disease cases. The high incidence of disease is a result of dramatic changes that have occurred in the landscape over the past half-century. Aerial photographs from 1926 until present demonstrate a change in landscape from 36% to 75% forest cover. Moreover, non-forested land use has changed from agriculture to residential, with moderate to large estates forming borders with mature deciduous forests. The emergence of Lyme disease, which is linked to the reintroduction of white-tailed deer and the increased abundance of other forest-dwelling wildlife, has placed an unusual proportion of the human population at risk because of residential exposure to infected tick vectors. Epidemiological studies of tick bites suggest that nearly 70% of Lyme disease victims receive infectious tick bites on their residential property. Random entomological surveys of endemic communities revealed the presence of infected nymphal ticks on 60-80% of individual properties.

In order to determine if Lyme disease risk can be predicted by the landscape characteristics of individual residential properties, we measured the areas of lawn, ornamentals, ecotone, and woods occurring

on each of 393 properties in two endemic villages by computer analysis of video images from aerial photographs. Nymphal tick densities were estimated by sub-sampling each landscape category with standard drag samples which were scaled for seasonal activity. Properties with a high proportion of wooded landscape were more likely to be tick infested, but this relationship is only weakly predictive as determined by poisson regression.

To determine the relationship between landscape features and Lyme disease risk at the community level, Landsat satellite data were used to classify residential landscapes and map landscape features characteristic of the residential-forest interface. The total amount of land area comprised of residential-forest interface was measured by GIS for each of the 25 municipalities comprising the county. Lyme disease risk for each municipality was measured by randomly sampling canine sera for antibodies to the Lyme disease spirochete, *Borrelia burgdorferi*. This single landscape feature was found to be highly predictive of Lyme disease risk (r=0.84).

Studies of landscape features by remote sensing and spatial analysis may provide a powerful tool for understanding the ecology and geographic distribution of Lyme disease risk in endemic areas.

STATUS OF HUMAN LYME DISEASE VACCINES

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Recent developments suggest that an effective vaccine for preventing human infection by the agent of Lyme disease will soon become widely available. Vaccination with recombinant outer surface protein A or B (rOsp) protects C3H mice from tick transmitted infection and disease. A novel mechanism of vaccine action appears to operate within the guts of vector ticks such that spirochetes fail to be transmitted, rendering redundant the classical antibody-mediated protection within the vaccinated host itself. Extensive studies in the C3H model suggest that antigenic heterogeneity of OspA does not negate protection conferred by rOspA derived from a single spirochetal isolate. Full protection is observed upon challenge with a variety of northeastern U. S., California, and European strains transmitted by tick bite. rOspB-B31 was fully protective against American strains, but failed in a challenge with a German strain. Phase I tests of a commercial rOspA preparation have been completed with no adverse reactions in 30 human subjects. A combined Phase II/III trial with 1000 subjects from 2 coastal New England sites is in advanced planning for 1994-1995. Lyme disease vaccines may be expected to play a significant role in protecting the public health in most American foci by the year 2000.

HUMAN AND CANINE BORRELIOSIS IN ALABAMA

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Human cases of Lyme disease have been documented in 44 counties in Alabama based on the case definition provided by the CDC. In a continuing effort to document the relative frequency with which different species of ticks attach to humans in Alabama, data have been compiled during the past four years (1990-1993) indicating that only five species are involved. *Amblyomma americanum* was by far the most frequently encountered (74% of total ticks). *Dermacentor variabilis* was a distant second (17%), followed

by *A. maculatum* (4%), *Rhipicephalus sanguineus* (3%) and *Ixodes scapularis* (2%). Three of these species have been found to be infected with spirochetes based on indirect fluorescent antibody (IFA) assays using monoclonal antibody H5332 for *Borrelia burgdorferi*: *A. americanum* (nymphs and males), *A. maculatum* (females) and *I. scapularis* (male).

A state-wide, random survey for canine borreliosis was conducted among veterinary practitioners in Alabama during 1991. A total of 579 serum samples was submitted from dogs that had not been vaccinated for Lyme disease, together with 428 ticks removed from the same dogs. Ten of the serum samples (1.7%) tested positive for antibodies to *B. burgdorferi* by IFA assay. The titers ranged from 1:64 in 2 dogs to 1:512 in 1 dog. The remaining 7 seropositive dogs had titers of 1:128. None of the dogs in the survey developed clinical signs of Lyme disease. Four species of ticks were submitted with the serum samples. *D. variabilis* was the most numerous (61%), followed by *R. sanguineus* (29%), *A. maculatum* (5%) and *A. americanum* (4%). Only one of the ticks tested positive for *B. burgdorferi*, an *A. maculatum* male.

A prospective study was conducted in rural Lee County, AL, to assess the exposure risk of dogs to infection with *B. burgdorferi* in an area where several human cases of Lyme disease have been documented previously. With the cooperation of 17 home owners, a total of 50 dogs was monitored over a two-year period (1991-1993). Eighteen of the dogs (36%) were monitored for 24 months, 8 (16%) for 19-22 months, 4 (8%) for 14-17 months, 4 (8%) for 12-13 months, 4 (8%) for 5-6 months, and 9 (18%) for less than 5 months. Blood samples were taken at monthly intervals, at which time any ticks on the dogs were collected. Sera were tested for antibody to *B. burgdorferi* by IFA. None of the dogs showed clinical signs of Lyme disease during the course of the study, and none of the dogs developed an antibody titer for *B. burgdorferi.* All ticks were identified to species and developmental stage. The ticks were then dissected and the midgut and salivary gland tissues examined for evidence of spirochetes by IFA assay (H5332). Over 800 ticks of the following species were collected and are listed in their order of abundance: *A. americanum, D. variabilis, I. scapularis, R. sanguineus* and *A. maculatum. A. americanum* comprised 56% of the collections compared to *I. scapularis* at 12%. Only *A. americanum* and *A. maculatum* were found to be infected with spirochetes.

Results from the state-wide survey and prospective field study indicate that the seroprevalence to *B. burgdorferi* in Alabama is low compared to some other regions of the United States. Together with the absence of any confirmed clinical cases of canine borreliosis in Alabama, there is little justification for recommending widespread use of canine vaccines to protect either dogs or humans from Lyme disease in the state.

LYME DISEASE SURVEILLANCE IN WESTERN KENTUCKY

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Lyme disease is the most commonly reported vector-borne disease in the United States based upon reports by state health agencies to the Centers for Disease Control (CDC). The disease was first recognized in the U. S. in 1975. In the northeastern and upper midwestern U. S., the bacterium responsible for the disease, *Borrelia burgdorferi*, is apparently maintained in a complex, multi-host cycle involving the black-legged tick, *Ixodes scapularis,* white-footed mouse, *Peromyscus leucopus*, and the white-tailed deer, *Odocoileus virginianus*. The number of human Lyme disease cases in the southeastern U. S. has steadily increased in recent years, but the vector species remains uncertain.

Lyme disease became a reportable disease in Kentucky in June 1985. From June 1985 through December 1990 the Kentucky Department for Health Services received 118 reports of the disease. Fifty-one of these met the current CDC case definition. More recently, from 1991 through 1992, 44 and 28 reported cases, respectively, met the case definition in Kentucky. Based upon this information it appears that Lyme disease transmission is occurring in the state.

In an attempt to determine whether the causative agent of Lyme disease, *B. burgdorferi*, is present in Kentucky a preliminary study was initiated. A total of 312 *P. leucopus* were collected and examined for antibodies against *Borrelia* sp. by an immunoblot technique using whole spirochete antigen. By this technique 21 of 312 (6.7%) samples were serologically positive. Each positive sample was tested further by western blotting against *B. burgdorferi* whole cell proteins. Eighteen (5.7%) of the samples possessed antibodies which bound to a 39/41 kDa protein band. Equal numbers of positive samples (9 and 9) were obtained from the eastern and western shores of Kentucky Lake. Forty ticks (*Dermacentor variabilis* larvae and nymphs) were removed from 24 of the 312 collected mice and examined by the indirect fluorescent antibody technique or the presence of spirochetes. All samples were negative. Mouse ear biopsies were collected and incubated in BSK medium, however *B. burgdorferi* was not recovered by this technique.

Based upon the serological indications from mice, a second study was undertaken which employed the polymerase chain reaction technique (PCR) in an attempt to establish which tick species harbored the spirochete, the geographic distribution, and the prevalences among tick species, males versus females, and nymphs versus adults. During 1992 and 1993 ticks were collected from raccoons, *Procyon lotor,* cottontail rabbits, *Sylvilagus floridanus*, and white-tailed deer, *Odocoileus virginianus*.
These ticks were analyzed for the presence of *B. burgdorferi* by PCR. Species-specific primers for a 309 bp portion of the *OspA* gene of *B. burgdorferi* were used in the amplification procedure. A total of 403 ticks of various species were identified and 1/2 of each tick was stored at -80 °C as a reference specimen for further analysis. The initial ticks analyzed consisted of pooled and unpooled (up to 5 ticks) samples for a total of 100 tests. A total of 36 samples were positive. Reanalysis of individual ticks from positive pooled samples plus unpooled resulted in an overall prevalence of 15.6% (63/403). Prevalence of *B. burgdorferi* by tick species was 14.8% (16/108) in *Dermacentor albipictus*, 30.5% (25/82) in *D. variabilis*, 10.3% (21/204) in *Amblyomma americanum*, and 11.1% (1/9) in *Haemaphysalis leporispalustris*. The tick species exhibiting the highest prevalence of *B. burgdorferi* was *D. variabilis*, all of which were removed from raccoons. The prevalence of the bacterium in female versus male ticks was only slightly higher (20% vs. 14%). A similar observation of prevalence was noted for adults versus nymphs (16% vs. 15%). The geographic distribution of infected ticks appeared to be random.

LYME DISEASE RESEARCH IN OKLAHOMA

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Lyme disease research in Oklahoma was initiated in 1989 in response to an increase in the incidence of reported cases in the southern regions of the United States. At that time, approximately 33 states had reported human cases of Lyme disease and Oklahoma had confirmed eight cases. Our objectives were to isolate, antigenically and geographically characterize *Borrelia burgdorferi* and finally, identify potential tick vector(s) of *B. burgdorferi.*

Initial efforts involved tick and rodent trapping from several regions of Oklahoma. *B. burgdorferi* was isolated from a field-caught *Peromyscus leucopus* from central Oklahoma. The strain was identified as *B. burgdorferi* by reaction with monoclonal antibody H5332, specific for the outer surface protein OspA of *B. burgdorferi.* This was the first isolation of *B. burgdorferi* from a wild mouse outside the normal range of the known vectors *Ixodes dammini* and *I. pacificus.*

Transmission of *B. burgdorferi* by laboratory reared *I. scapularis*, *Dermacentor variabilis* and *Amblyomma americanum* was evaluated by feeding each tick species on New Zealand white rabbits experimentally infected with *B. burgdorferi* (JDI strain). At repletion, spirochetes could be detected by dark-field microscopy only in *I. scapularis*. Acquisition rates were 18 and 21%. When previously exposed nymphs of each species were fed on susceptible rabbits, *I. scapularis* was the only tick of the three species that transmitted *B. burgdorferi*. When a single rabbit was experimentally infected with *B. burgdorferi* and infested at 7-d intervals with *I. scapularis*, *A. americanum*, *D. variabilis*, and a second time with *I. scapularis*, *B. burgdorferi* was detected again only in cultures from the two groups of *I. scapularis*. When molted nymphs from each tick species were allowed to feed on susceptible rabbits, spirochetes again were isolated only at necropsy from the rabbits on which the two groups of *I. scapularis* fed.

B. burgdorferi was isolated from *I. scapularis* and *Dermacentor albipictus* that were removed as partially fed adults from white-tailed deer in Oklahoma. Isolation in media was accomplished only after homogenates of pooled field-collected ticks were inoculated into laboratory reared *P. leucopus* and reisolated from the urinary bladder into BSK-II media. Both isolates were confirmed by western blot analysis and reactivity with monoclonal antibody H5332. These are the first reported isolates of *B. burgdorferi* from Oklahoma from these two tick species and are the first isolates from ticks in the south-central U. S. that were infective for laboratory reared *P. leucopus*.

Methods to recover *B. burgdorferi* from living *P. leucopus*, *P. maniculatus* and *Reithrodontomys fulvescens* were tested. Needle aspiration proved to be better than either ear punch biopsy or blood inoculated media (BSK-II). Also, *R. fulvescens* was first documented as an experimental host and may play a role in the epidemiology of Lyme disease.

A serological survey of 223 dogs (1989 collections) and 489 white-tailed deer (1975-90 collections) were tested for antibodies to *B. burgdorferi* using an indirect kinetic ELISA. Twenty-six dogs (11.7%) and 22 deer (4.5%) samples were positive. Deer reactors were first detected among 1978 samples taken in central and eastern Oklahoma, but reactive dogs were mostly from central Oklahoma. Confirmed human cases of Lyme disease between 1986 and 1989 were distributed throughout the state, thus showing no correlation with either deer or dog results.

TICKS, THEIR HOSTS, AND *BORRELIA BURGDORFERI* ON THE OUTER BANKS OF NORTH CAROLINA

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During a preliminary survey, adult *Ixodes scapularis* were collected at five sites on the Outer Banks of North Carolina. Isolates of *Borrelia burgdorferi* were obtained from ticks collected at three locations, Buxton Woods, Coquina Beach and Pine Island. These initial isolates prompted additional sampling along the Outer Banks to: 1) obtain additional isolates of *B. burgdorferi*; 2) estimate *B. burgdorferi* prevalence in ticks; and 3) determine tick-host associations. Twenty-nine additional isolates of *B. burgdorferi* were made from adult *I. scapularis* collected between November 1991 and August 1993 at Pine Island, Buxton Woods and Coquina Beach. More than 20% of the *I. scapularis* adults collected at Pine Island and examined by indirect fluorescence microscopy (IFA) with a species-specific monoclonal antibody (H5332) were infected with the spirochete. No spirochetes were observed in more than 1,300 *Amblyomma americanum* collected at Pine Island and Buxton Woods.

Rodents and lizards were intensively live-trapped at the Pine Island and Buxton Woods study sites. At Pine Island, rodents were sampled in traps placed in lines and grids, and lizards were trapped using drift-fences fitted with funnel traps, pit fall traps and coverboards. At Buxton Woods, rodents were sampled using traps that were placed in grids, and lizards were trapped with drift-fences fitted with funnel traps. White-footed mice (*Peromyscus leucopus*) (n=126) and rice rats (*Oryzomys palustris)* (n=69) were the most frequently trapped rodent hosts. Immature *I. scapularis* larvae were found on both hosts (mean = 0.11, SD = ± 0.54, 0.58 ± 1.8 /rodent, respectively) but nymphs were proportionately more numerous on rice rats (30%) (0.32 ± 0.63/rat) than on white footed mice (6%) (0.05 ± 0.22/mouse). Although house mice (*Mus musculus*) were also collected, they were less numerous (n=8) and harbored no *I. scapularis.* Isolates of *B. burgdorferi* were obtained from both white-footed mice and rice rats. The six-lined race runner, *Cnemidophorus sexlineatus*, was the only lizard trapped at Pine Island. Larvae were found on 7% (0.12 ± 0.48/lizard) of the race runners trapped and 3% (0.02 ± 0.14/lizard) of the animals examined harbored nymphs. *Peromyscus* (n=284) were the most frequently trapped rodents at Buxton Woods; few rice rats (n=7) and house mice (n=26) were trapped. Although larvae were found on 13% of the white-footed mice trapped, nymphs were only found on 1%. No nymphs were found on the rice rats and house mice. Four species of lizards were trapped at Buxton Woods; the six-lined race runner (n=57) and the ground skink (*Scincella lateralis*) (n=38) were the most frequently collected lizards. Larval *I. scapularis* were found on 1% of the race runners and 11% of the ground skinks; however, no nymphs were found on lizards of either species.

Marsh rabbits (*Sylvilagus palustris*) (n=82) and eastern cottontails (*S. floridanus*) (n=19) were trapped at the Pine Island site using wooden box-traps. Four species of ticks *A. americanum, Haemaphysalis leporispalustris, I. dentatus* and *I. scapularis* were found on these hosts. *H. leporispalustris* was the most frequently collected tick from both species of hares. *I. scapularis* larvae were more frequently collected from *S. floridanus* (16%) (0.26 ± 0.73/host) and *S. palustris* (9%) (0.22 ± 0.88/host). Nymphs were more frequently observed on *S. palustris* (16%) (0.05 ± 0.05/host), than *S. floridanus* (5%) (0.16 ± 0.43/host) Two isolates of *B. burgdorferi* were obtained from hares.

Based on these studies, we conclude that: 1) *B. burgdorferi* is present in *I. scapularis* at several sites on barrier islands that comprise the Outer Banks of North Carolina; 2) white-footed mice serve as important hosts for immature *I. scapularis* at Pine Island and Buxton Woods; 3) rice rats serve as an alternative host and potential reservoir of *B. burgdorferi* at Pine Island, but are less important as hosts for *I. scapularis* at Buxton Woods; and 4) lizards serve as hosts for *I. scapularis* at both sites but their role as hosts varies with the abundance and diversity of rodent species available as hosts; and 5) hares serve as alternative hosts for immature *I. scapularis* at Pine Island and potentially support a secondary cycle of *B. burgdorferi* transmission.

TICK-HOST ASSOCIATIONS AND MAINTENANCE OF *BORRELIA BURGDORFERI* IN VIRGINIA

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The ecology of Lyme borreliosis was compared during a 2-year period (1991 - 1993) in two localities in Virginia. These included a coastal site on Assateague Island, along the Atlantic coast, and an inland site along the York River near Williamsburg. The coastal site was comprised of bayberry thickets, grassy meadows and brackish marshes. *Borrelia burgdorferi* was isolated from 13 small mammals in this site,

including 3 white-footed mice (*Peromyscus leucopus*), 6 feral house mice (*Mus musculus*), 3 rice rats (*Oryzomys palustris*) and a least shrew (*Cryptotis parva*), from a total of 323 animals sampled. No isolations were made from samples from 15 meadow voles (*Microtus pennsylvanicus*), 7 opossums (*Didelphis virginiana*) and 15 raccoons (*Procyon lotor*). Serologic assays (ELISA, Western Blot) indicated that approximately 47% of the feral house mice and 30% of the white-footed mice in the site had been exposed to *B. burgdorferi*. The black-legged tick, *Ixodes scapularis* (=*I. dammini*), is relatively abundant in the area. No isolations were made from more than 265 larvae from small mammal hosts examined by the direct immunofluorescence antibody assay (IFA) using a monoclonal antibody against *B. burgdorferi* OspA. However, approximately 15% of 89 nymphs and 36% of 13 adults sampled in the study site had spirochetes detected by IFA. During the seasonal activity period, black-legged tick larvae were numerous on white-footed mice (2.39 larvae/mouse, 76.3% of larvae collected), but were also found on feral house mice (0.37 larvae/mouse, 16.6% of larvae collected), rice rats (0.80 larvae/rat, 6.6% of larvae collected) and least shrews (0.08 larvae/shrew, 0.6% of larvae collected). Nymphal ticks were dispersed among a wider variety of hosts, including opossums (*D. virginianus*), meadow voles (*M. pennsylvanicus*) and raccoons (*P. lotor*), as well as the same 4 small mammals noted above. White-footed mice supported the largest percentage of nymphal ticks collected (0.42 nymphs/mouse, 39.9% of nymphs collected), but feral house mice (0.20 nymphs/mouse, 29.5% of nymphs collected) and rice rats (0.64 nymphs/rat, 17.2% of nymphs collected) were important secondary hosts.

At the inland site near Williamsburg, Virginia, *B. burgdorferi* was isolated from 2 white-footed mice from a total of 260 animals sampled. No isolations were made from numerous specimens of 8 other species. Serologic assays showed that 17% of the white-footed mice had antibodies to *B. burgdorferi*. Black-legged ticks, *I. scapularis* (=*I. dammini*) appeared to be considerably less abundant at this locality than at the Assateague Island coastal site. No evidence of *B. burgdorferi* was found among the 35 larvae, 18 nymphs and 259 adults examined (IFA) for specimens collected at this site. White-footed mice were the most important hosts for immatures of this tick. These mice supported 86.2% of all larvae collected (0.40 larvae/mouse) and 71.4% of all nymphs collected (0.08 nymphs/mouse).

If the two study sites are representative of natural conditions, these studies may indicate that Lyme borreliosis is more prevalent along the Atlantic coast than in inland areas. Isolations were much more numerous and seroprevalence much higher at the coastal site than at the inland site. In addition, *I. scapularis* (=*I. dammini*) larval and nymphal density, based on evidence from small mammal samples, was higher (6.0 and 4.6 times, respectively) at the coastal site than at the inland site. Consequently, the risk of human exposure to Lyme Disease may be higher in the coastal area than elsewhere in Virginia.

TICK-RACCOON INTERACTIONS IN THE COASTAL PLAIN OF NORTH CAROLINA

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In 1988 and 1989, a marked increase occurred in the number of cases of Lyme disease that were reported from Marine Corps Base, Camp Lejeune, NC. We initiated a comprehensive study of the epizootiology of Lyme disease on the Marine Corps Base in 1990. The principle objective of our study was to determine the tick vector(s) and wildlife reservoir(s) of *Borrelia burgdorferi*.

From July 1990 to May 1992 a variety of species of wildlife including snakes, lizards, birds and mammals that were either live-trapped or killed were examined for ticks. A total of 91 rodents including 76

Peromyscus spp., 244 raccoons, 507 white-tailed deer, small numbers of black bears, horses, dogs, and foxes, over 300 birds representing approximately 50 species, 95 snakes representing 16 species, and 148 lizards representing 7 species were examined. Host-associated ticks representing six species were tested for infection with the Lyme disease spirochete by an indirect immunofluorescence assay (IFA) using *B. burgdorferi*-specific monoclonal antibody H5332. Small numbers of spirochete-infected ticks were recovered from hosts but the majority of these infected ticks were removed from raccoons (*Procyon lotor*). In addition, 176 (72.1%) of 244 raccoons examined were found to be infested with ticks. These results suggested the possible involvement of the raccoon as a reservoir host for *B. burgdorferi* on the Marine Corps Base.

Accordingly, from May 1992 to July 1993, field work was focused on studies of the raccoon population on the Marine Corps Base. Our objectives were: 1) to estimate the density of raccoons in representative habitats; 2) to determine seasonal changes in species composition and numbers of ticks of each species parasitizing raccoons; 3) to ascertain the incidence and species identification of *B. burgdorferi*-infected ticks infesting raccoons; and 4) to determine the prevalence of *B. burgdorferi*-infected raccoons.

The majority of the raccoons (207/231) that were live-trapped were found to be infested with ticks. Seven tick species were collected from these animals. *Amblyomma americanum* was the predominant species, infesting 80.2% (166/207) of the raccoons. *Dermacentor variabilis*, *Ixodes texanus*, and *Ixodes scapularis* infested 36.8% (75/207), 33.3% (69/207), and 24.7% (51/207) of the raccoons examined, respectively. The majority of ticks were found to be attached on the head and in areas where the pelage was thin. *A. americanum* were primarily attached on the head (50.4%), followed by the hind quarter (21.8%), shoulder (15.1%), and torso (12.6%). *D. variabilis* (90.8%), *I. texanus* (74.4%), and *I. scapularis* (98.6%) were predominantly removed from the head.

An IFA was used to test 3,298 of 16,523 host-associated ticks, removed from raccoons and opossums (*Didelphis virginiana*), for infection with *B. burgdorferi*. Three species, *A. americanum* (30/2,937), *I. texanus* (11/271), and *D. variabilis* (2/90), were found to be infected.

To determine the prevalence of spirochete-infected animals, blood samples taken from 81 raccoons were added to BSK-II spirochete culture media. Spirochetes were cultured from 22 (27.2%) of the 81 blood samples taken from raccoons. Culture-positive animals were trapped in winter and early spring months from December, 1992 to April 1993. The majority of these isolates have not been identified; however, some isolates have been confirmed to be *B. burgdorferi* by IFA with a species-specific monoclonal antibody (H5332) and by PCR. Because of the small number of spirochete-infected ticks removed from raccoons (some of which were culture-positive), we conclude that the raccoon is an inefficient reservoir for *B. burgdorferi*.

RESERVOIR COMPETENCE OF THE RACCOON (*PROCYON LOTOR*) FOR THE LYME DISEASE SPIROCHETE, *BORRELIA BURGDORFERI*

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Attempts were made to infect five raccoons by exposing them to *Borrelia burgdorferi*-infected *I. dammini* nymphs. Larval or nymphal *I. dammini* were used to detect infection through xenodiagnosis. Ticks were examined by a immunofluorescence antibody assay for spirochete infection. Blood samples were taken at approximate weekly intervals, and a skin biopsy was taken from each animal every third week throughout the study. Spirochetal infection was monitored by placing blood and skin biopsies in BSK-II

spirochete culture media. Raccoon antibody response to *B. burgdorferi* was examined using enzyme-linked immunosorbent assay and immunoblot techniques.

Spirochetes were infrequently isolated by culture of skin and blood from one raccoon and skin from a second raccoon. Spirochetes were not recovered though xenodiagnosis. Raccoons expressed both homospecific and heterospecific tolerance against tick feeding. With exception of one raccoon, experimental animals did not exhibit an antibody response to *B. burgdorferi* until they were fed upon by a second cohort of spirochete-infected *I. dammini* nymphs. Western blot analysis indicated that raccoons infected via tick bite generally responded to the 31- (OspA) and 34-kDa (OspB) epitopes of *B. burgdorferi* and that individual response to other epitopes was variable.

Although spirochetes were recovered from the skin and blood of some raccoons, ticks were not infected by feeding on these animals. To infect attached ticks, raccoons may require more frequent and prolonged exposure to infected ticks than they received in this study. Our results suggest that although some raccoons are susceptible to infection, they are not reservoirs of *B. burgdorferi*.

RESERVOIR COMPETENCE OF THE RICE RAT AND LIZARDS FOR THE LYME DISEASE SPIROCHETE, *BORRELIA BURGDORFERI*

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Part I. Reservoir competence of the Rice Rat (*Oryzomys palustris*) for *Borrelia burgdorferi*.

In areas of the Northeastern United States where Lyme disease is prevalent, the white-footed mouse (*Peromyscus leucopus*) serves as the principal host for *Ixodes scapularis*, the primary vector of *Borrelia burgdorferi*. Due to its efficiency in infecting attached ticks and its relative abundance, it also serves as the predominant reservoir of the spirochete. In the Southeast, *P. leucopus* densities are relatively low compared to those in the Northeast. Marsh rice rat (*Oryzomys palustris*) coexists with *P. leucopus* throughout the Coastal Plain of North Carolina. *B. burgdorferi* has been detected in ticks removed from rice rats in Coastal Virginia suggesting host infection with the spirochete. Accordingly, we evaluated the reservoir competence of rice rats for *B. burgdorferi*.

In particular, we assessed the susceptibility of rice rats to infection with the spirochete, their ability to infect attached ticks and duration of infectivity. Rice rats were infected either by subcutaneous inoculation of spirochete-containing suspensions or by exposing them to infected *I. scapularis* nymphs. Tick-naive, *Borrelia*-free golden Syrian hamsters served as control animals for all our experiments. Cohorts of infected ticks for exposing rats to *B. burgdorferi* were produced by feeding them on Syrian hamsters infected with a New York State strain. The infectivity of this particular strain of *B. burgdorferi* for hosts and ticks was demonstrated by reisolation of the spirochetes from hamsters and by detecting their presence in newly molted nymphs. *B. burgdorferi* isolated from the infected hamsters (the first passage) were used for subcutaneous inoculation of four rice rats. Infected nymphs, obtained by feeding *I. scapularis* larvae on infected hamsters, were used for the infectious feeding on six rice rats. *B. burgdorferi* infection in experimental and control animals was determined by xenodiagnosis and by the culturing of tissues in BSK-II medium. All positive results were confirmed by indirect fluorescence antibody assay (IFA) with a *B. burgdorferi* species-specific monoclonal antibody (H5332).

Both injected and tick-exposed rice rats acquired *B. burgdorferi* and maintained spirochete infection for five to nine weeks. *B. burgdorferi* were found in samples of skin and urinary bladders from all animals

killed on day 8, 21, 35 or 63 post exposure. *B. burgdorferi* were detected in blood samples of four tick-exposed rats one week after exposure, and in the blood of three rats at the end of the second week. The concentration of spirochetes in the blood of some infected rice rats may have been too low to be cultured. Spirochetes cultured from rice rats were infectious for hamsters.

Rice rats infected with *B. burgdorferi* transmitted the agent to feeding *I. scapularis* larvae. An average of 2.7% of nymphs (n=482), fed as larvae on injected rats, became infected. Tick-exposed rice rats provided an average infection rate as high as 75.6% in nymphs (n=694). These animals became infectious for ticks at the end of the first week after attachment of infected nymphs. Ticks fed during the second to fourth weeks had the highest infection rate - up to 85%. About 68% of xenodiagnostic ticks acquired *B. burgdorferi* from tick-exposed rice rats during the fifth week, and 17.4% during 9th week. *I. scapularis* nymphs infected by feeding on rice rats were able to transmit infection to naive rats. Two rice rats were infected after being infested with nymphs derived from xenodiagnostic larvae. *B. burgdorferi* were isolated from skin and urinary bladder samples of both of these rats three weeks after the infectious feeding.

Thus, the rice rat appears to be reservoir competent for *B. burgdorferi*. Its susceptibility to spirochetes and ability to infect ticks indicates that the rice rat may play an important role in dissemination and maintenance of the Lyme disease agent in its natural circulation in the southeast.

Part 2. Reservoir competence of the southeastern five-lined skink (*Eumeces inexpectatus*) and the green anole (*Anolis carolinensis*) for *Borrelia burgdorferi*.

A wide range of vertebrate species serve as hosts for the primary vector of *B. burgdorferi*, the black-legged tick (*Ixodes scapularis*). In the Coastal Plain of North Carolina, various species of lizards serve as hosts for *I. scapularis* larvae and nymphs, in contrast to rodents which are infrequently parasitized by ticks. So far, only the western fence lizard from California and the sand lizard from Europe have been tested and found to be reservoir incompetent for *B. burgdorferi*. However, the susceptibility of various species of reptiles to infection is likely to be as different as it is for mammalian species. Accordingly, we evaluated the reservoir competence of southeastern five-lined skink (*Eumeces inexpectatus*), and green anole (*Anolis carolinensis*) for *B. burgdorferi*.

In particular, we assessed the susceptibility of these two species of reptiles to infection with the spirochete and their ability to infect attached ticks. Basically, the experimental design employed in reservoir competence trials of rice rats was used. Lizards were infected either by subcutaneous inoculation of spirochete-containing suspensions or by exposing them to infected *I. scapularis* nymphs. *B. burgdorferi* infection in experimental animals was determined by xenodiagnosis and by culturing of tissues in BSK-II medium. All spirochetes were confirmed to be *B. burgdorferi* by IFA with monoclonal antibody H5332.

Both skinks (n=6) and anoles (n=6) inoculated with about 10^6 spirochetes, contained in 0.1 ml of suspension, were able to acquire *B. burgdorferi*. Tissues evaluated on day 31 after inoculation yielded positive results. Spirochetes, detected by dark-field microscopy in tissue cultures of two injected skinks and two anoles, were confirmed as *B. burgdorferi* by IFA. The agent was reisolated from blood, kidney and liver samples of these lizards.

Feeding of xenodiagnostic ticks was initiated on the fourth day after inoculation. Twenty-five to thirty *I. scapularis* larvae were placed on each injected lizard twice per week during four consecutive weeks after inoculation. In this way, each lizard was continuously parasitized by ticks for five to six weeks. Recovery of ticks was low because both species readily eat flat and especially engorged ticks. *B. burgdorferi* were found in two of 146 ticks that engorged on southeastern five-lined skinks and two of 91 that fed on anoles.

Infected nymphs (five per animal) were placed on 6 naive southeastern five-lined skinks and six naive anoles. *B. burgdorferi* spirochetes were found in tissues of all tick-exposed southeastern five-lined skinks and all tick-exposed anoles after euthanasia was done on days 35 and 41, respectively. Both southeastern five-lined skinks and anoles readily acquired *B. burgdorferi* from infected *I. scapularis* nymphs and maintained the agent in their bodies for at least five to six weeks. Positive cultures were obtained from blood samples of five southeastern five-lined skinks and one anole; skin of four southeastern five-lined skinks and one anole; kidneys of five southeastern five-lined skinks and two anoles; livers of four

southeastern five-lined skinks and all five anoles; and even from the large intestines of two southeastern five-lined skinks and three anoles.

Two isolates recovered from the skin of one southeastern five-lined skink and from the liver of one anole were tested for infectivity by subcutaneous inoculation to hamsters after one passage in BSK-II medium. Hamsters were killed on day 21 after inoculation and the agent was successfully reisolated from their skin, urinary bladder and liver samples.

For xenodiagnosis, tick-exposed lizards were continuously infested with *I. scapularis* larvae for five to six weeks after the feeding of infectious ticks. In total, 22.6% of 221 newly molted nymphs recovered from tick-exposed southeastern five-lined skinks were infected with *B. burgdorferi*. In contrast, only one of 47 (2.1%) nymphs recovered from tick-exposed anoles was infected with *B. burgdorferi*.

Thus, both southeastern five-lined skinks and green anoles are susceptible to *B. burgdorferi* infection. They acquire spirochetes and maintain *B. burgdorferi* infection for at least one month. Southeastern five-lined skinks can infect up to 23% of attached ticks, while green anoles infect about 2% of attached ticks. Because of their susceptibility to the agent, southeastern five-lined skinks should be able to participate in dissemination of *B. burgdorferi* and in its maintenance in natural circulation. Green anoles are not effective in transmission of *B. burgdorferi*. They also do not serve as common hosts for the tick vectors under field conditions.

ECOLOGY OF *IXODES SCAPULARIS* IN THE SOUTHEAST

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As part of an ongoing study, *Ixodes scapularis* populations were sampled from November 1992 through August 1993 on St. Catherine's Island, Georgia. Previous data have shown that *I. scapularis* is abundant on this barrier island and that cases of Lyme borreliosis have been reported by visitors. Ticks were sampled by three methods: quantitative dragging/flagging, live-trapping mammals, and noosing and live-trapping reptiles. Questing adult *I. scapularis* were collected from November through May with a peak population density in February. Questing larvae were recovered from May through August with a peak in May, while questing nymphs were present from April through August with a peak in July. Larval *I. scapularis* were collected from live-trapped cotton mice in every sample month with a sharp peak in June (93% prevalence, mean intensity = 3.0), while nymphs were collected from April through August with a current peak in August (20% prevalence, mean intensity = 1.3). Seven species (51 individuals) of reptiles were noosed or pit-fall trapped from April through August. Two species of snakes (*Thamnophis sauritus* and *T. sirtalis*), the green anole (*Anolis carolinensis*), and the ground skink (*Scincella lateralis*) were not infested by ticks. However, the southeastern 5-lined skink (*Eumeces inexpectatus*), the broad-headed skink (*Eumeces laticeps*), and the island glass lizard (*Ophisaurus compressus*) were all heavily infested with immature *I. scapularis*. Every individual of these three species of lizards was infested. The highest mean intensity of *I. scapularis* larvae (28.5) was found on *E. inexpectatus* and of nymphs (28.0) on *O. compressus*. The 5-month lizard samples did not permit an analysis of the phenology of immature *I. scapularis* on these hosts but a separate study of 889 museum-preserved lizards (8 species) from four southeastern states showed a peak of both larval and nymphal ticks in May. We believe that our data reflect a poorly-regulated life-cycle for *I. scapularis* on St. Catherine's island with a 1-year life-cycle predominating but with some 2-year cohorts. The peak mean intensities for larval and nymphal ticks on infested lizards were approximately 10-fold and 20-fold, respectively, higher than comparable intensities on cotton mice. However, because only the cotton mice may be reservoir-competent for *Borrelia burgdorferi*, the larger numbers of ticks on reptiles probably affects the epidemiology of Lyme borreliosis on the island. Isolates of *B. burgdorferi* were made from 3 of 39 cotton mice. Data on seasonal tick infestation, average

tick feeding times, cotton mouse population densities, and the proportion of spirochetemic mice, allowed the total number of annual infective (nymphal and adult) *I. scapularis* for the island to be estimated at a minimum of 126,910 ticks. Data from this study may be representative for the Southeast, particularly in coastal regions.

CHEMICAL CONTROL OF TICKS ON WHITE-TAILED DEER

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Ixodicide-medicated baits and topical treatment devices are being tested as methods to effect efficacious and cost-effective control of ticks on white-tailed deer, and, thereby, reduce the risk to humans and livestock of infection with tick-transmitted disease agents. For the second successive year in an area with a dense population of *Amblyomma americanum*, one of two groups of deer confined in adjacent pastures have been provided whole-kernel corn medicated with ivermectin. The medicated corn has been made available to deer daily from February through September. In 1992, over a 37-day interval that began 62 days after initiation of treatment, blood samples were collected from 6 does and 1 buck that were captured with tranquilizing darts. The mean concentration of ivermectin in blood serum samples was 21.7 (±3.02) ppb. Serum collected from deer after curtailment of treatment showed that the serum concentration of ivermectin dropped to undetectable levels (<2 ppb) within 28 days. In 1993, serum from 4 does and 2 bucks had a mean concentration of 30 (±19.14) ppb ivermectin. No engorging adult ticks were found on any of the treated deer sampled during 1992 and 1993, while untreated animals sampled from the control pasture were heavily infested with engorging adults. The degree to which the tick population is being controlled by the treatment is being measured by comparing the indices of abundance of free-living host-seeking ticks. These indices were being obtained by comparing, in the treated and untreated pastures, values from systematic sampling of adults and nymphs with CO_2 traps and sampling of larvae collected with a "flip-cloth" method. Based on samples collected from 01/08/93 through 08/18/93, 83% of adult ticks, 92% of nymphs, and 100% of larvae have been controlled in the treated pasture.

Several passive topical treatment devices are in various stages of development. Such treatment devices are designed so that deer are attracted by a bait such as whole-kernel corn and incidentally treat themselves topically with an ixodicide when they contact an ixodicide-coated surface. The device currently being tested has a centrally located bin that will hold 45 kg of corn. Gravity dispenses small quantities of corn into a tray at either end of the device. Two 30.5 cm ixodicide-coated rollers are positioned on each end, and deer are treated as they feed on the corn. Two methods currently are being developed and tested for applying and maintaining appropriate quantities of ixodicide on the rollers. One approach is to fix amitrax-impregnated plastic strips to the outside of each roller. The second method involves spring-activated valves that allow liquid ixodicide to be replenished on the surface of rollers each time deer use the device. Results of preliminary tests of these designs in the field indicate that one or both versions of the device may provide safe, effective, and relatively inexpensive control of ticks on white-tailed deer.

DISTRIBUTION OF *BORRELIA BURGDORFERI* AT DOD FACILITIES

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These data were collected by past and present staff of the Army Environmental Hygiene Activity-North. We are indebted to the assistance of numerous DoD entomologists, preventive medicine specialists, and especially to our former Chief, LTC Harold J. Harlan. The data were collected in the course of providing assistance to installation commanders and managers in assessing the risk from Lyme disease to troops training at facilities, to personnel and dependents engaged in recreational activities, and to installation employees or visitors. Data were collected at installations in an 18 state area spanning from North Carolina westward to Indiana and north to the Canadian border. Other southeastern states were surveyed by our sister laboratory at Fort McPherson, Georgia.

Data were collected in several ways. Installations that had deer hunting seasons were visited during the hunt, when ticks and deer sera were collected at the hunt control check stations. Where there were no deer hunts, small and medium sized mammals were trapped, and ticks were collected. In addition miscellaneous ticks were collected, for example, from working dogs, from pets by veterinarians, or via tick drags. Ticks were also submitted for testing by various health clinics. Tick gut contents have been analyzed since 1986 for the presence of *Borrelia burgdorferi* using polyclonal and monoclonal DFA or IFA procedures depending on availability of conjugate.

Low, moderate, or high risk categories were ascribed to installations based on a non-statistical evaluation of qualitative and quantitative information available on Lyme disease locally. To the extent available, information evaluated included the following elements:

(1) History of Lyme disease in the area.

(2) The presence of the tick vector (*Ixodes scapularis*) and the host population needed to sustain a viable population of the vector.

(3) The presence of the Lyme disease-causing spirochete (*B. burgdorferi*) in the tick population.

Once gathered, this information was used to ascribed risk in the following manner:

Low-Some risk elements identified in nearby areas, not locally.

Moderate-Some risk elements identified from the installation, or human Lyme disease cases reported locally.

High-All risk elements present on the installation.

LOW:	MODERATE:	HIGH:
Crane AAP, IN	Dahlgren NSWC, VA	Ft. Belvoir, VA
Ft. Drum, NY	Ft. Devens, MA	Ft. Bragg, NC
Cp. Grayling, MI	Ft. Indiantown Gap, PA	Ft. Dix, NJ
Ft. Knox, KY	Indian Head NSWC, MD	Earle NWS, NJ
Lexington-Blue Grass AD, TN	Sunny Point MOT, NC	Cp. Edwards, MA
	Ft. Pickett, VA	Ft. Eustis, NJ
Letterkenny AD, PA	Quantico, VA	Ft. Hill, VA
Seneca AD, NY	Ft. Ritchie, MD	Ft. Meade, MD

Tobyhanna AD, PA	Cp. Peary, VA
Woodbridge, VA	Picatinny A, NJ
	Cp. Ripley, MN
	Lakehurst, NJ
	Cp. Smith, NY
	Stones Ranch, CT

Data collected over time have recorded changes in the distribution and abundance of *I. scapularis*. At Ft. A. P. Hill, for example, surveillance was begun in 1986. A single *I. scapularis* was collected from 64 deer examined in 1988. The population gradually increased through 1992, when 259 *I. scapularis* were collected from 39 of 52 deer examined.

A comparison of spirochete infection in *I. scapularis Dermacentor albipictus*, and *Amblyomma americanum* with deer serology results reveals inconsistencies in expected results. At Camp Peary and Ft. A. P. Hill, deer serology does not confirm infection observed in ticks. At Ft. Pickett and Quantico testing on *D. albipictus, A. americanum* and deer serology indicate spirochete presence, but *I. scapularis* test negative. At Ft. Bragg there is indication of *Borrelia* spp. in *I. scapularis* while other species of ticks and deer test negative.

Data also indicate infection rates observed in ticks from deer may underestimate infection rates in host-seeking ticks, and therefore risk evaluation. At Camp Edwards, the infection rate for *B. burgdorferi* in host-seeking ticks was 40% higher than for ticks collected from deer (HS n=253, %+=56; D n=845, %+=16; X^2 test, $P<0.001$).

In summary, we have noted that over time a greater number of installations are at risk for Lyme disease, that deer check station surveillance is the most productive and cost effective survey tool, that the role and consequence of *D. albipictus* and *A. americanum* in *B. burgdorferi, Borrelia* spp., and other pathogens (e.g., *Ehrlichia*) transmission is uncertain.

DISTRIBUTION AND CHARACTERIZATION OF *BORRELIA BURGDORFERI* ISOLATES FROM GEORGIA AND FLORIDA

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A total of 20 *Borrelia burgdorferi* isolates have been cultured from cotton mice (*Peromyscus gossypinus*), cotton rats (*Sigmodon hispidus*), and the ticks *Ixodes scapularis* and *I. dentatus* from Georgia and Florida. One isolate from a human has also been cultured, but it is unclear whether the infection was obtained in Georgia, Holland, or Sweden. Comparisons of isolates from these three localities are in progress.

Spirochetes were cultured from rodents and ticks from four geographic localities in Georgia. Two locales were barrier islands (Sapelo and St. Catherines), one locale was coastal mainland (St. Marys), and one locale was from the fall line of the coastal plain and piedmont regions (Bibb County) in the center of the state. In Florida, *B. burgdorferi* were obtained from three geographic localities including Merritt Island, Faver-Dykes State Park (coastal plain), and Amelia Island. Foci from the coastal region of the two states from which *B. burgdorferi* were cultured extend for approximately 300 miles (Savannah, Georgia, to the Cape Canaveral, Florida, area). The Bibb County site in central Georgia is approximately 200 miles inland from the Georgia coast. Lack of adequate sampling prohibits statements as to limits of *B. burgdorferi* distribution in Georgia and Florida.

Peromyscus gossypinus and *Sigmodon hispidus* represent new host records for *B. burgdorferi*. It is likely that both species serve as reservoirs. Wild-caught, infected *S. hispidus* brought into the laboratory remain infected for at least 12 months. Although small samples of other species of small and medium-sized mammals have not yet yielded cultures of *B. burgdorferi*, serological data indicate that variously sized mammals of several species were exposed to the spirochete.

The spirochete isolates were identified as *B. burgdorferi* by positive reactivities to two outer surface protein A (Osp A H3TS, H5332), two Osp B (H5TS, H6831), and *Borrelia* genus-specific (H9724) monoclonal antibodies; negative reactivities were recorded from *Borrelia hermsii* (9826) and *Borrelia coriaceae* (F6F3, F6B3, F6B11) monoclonal antibodies. Additional characterizations were obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of whole spirochetal lysates. The polymerase chain reaction was also used to detect several known DNA target sequences specifically found in *B. burgdorferi* reference strain B-31.

ATTEMPTS TO CULTURE *BORRELIA BURGDORFERI* IN MISSOURI

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One of the criteria for demonstrating that a particular arthropod is the vector of a pathogen is to culture the pathogen and then demonstrate that the proposed vector can transmit the pathogen in an infective state. *Borrelia burgdorferi* has been grown in BSK-II media by a number of investigators and from a variety of sources on a rather regular basis in the northeast, northcentral, and western parts of the U. S. In contrast, it has been very difficult to culture the spirochetes from the lone star tick (*Amblyomma americanum*) and the American dog tick (*Dermacentor variabilis*) in the southern half of the U. S. The spirochetes from these two species of ticks in Missouri have been shown to have certain conserved genomic regions that are highly homologous to the B31 strain of *B. burgdorferi* on the basis of DNA and rRNA analyses. The spirochetes from the lone star tick and the American dog tick react with the monoclonal antibody to OspA of *B. burgdorferi* (H5332). Why has it been so difficult to culture the spirochetes from Missouri? Does the inability to culture the spirochetes mean that they are not *B. burgdorferi* and therefore cannot cause Lyme disease as some people are claiming?

The definition of a species has plagued biologists for a long time. To some, the definition of a species was the absence of fertile offspring. However, the dog, coyote, and wolf in any combination can produce fertile offspring although they are different species or even different genera. There are examples of successful breeding in the laboratory but no indication of it in the wild; are these to be considered the same species? DNA and rRNA homologies have become important criteria for species identity although not everyone agrees on the percent homology necessary for species identity. There are other definitions of species of course. What makes a subspecies? What makes a strain? These are man-made terms designed to make a useful organization of names but they carry many connotations. Can different species of microorganisms cause the same clinical disease? The literature indicates that they can.

Is it possible that two strains of the same species (by one definition or another) from different habitats can have different nutritional requirements? Or would that make them subspecies? It seems quite reasonable to me that spirochetes from the lone star or American dog tick would be a different strain from spirochetes from an *Ixodes* species. Therefore, I have been making a number of modifications in the BSK-II media and trying to grow spirochetes from various sources in Missouri and southern Illinois. I have made 17 different media and tried culturing specimens from ticks, small mammals, and humans. Unfortunately, I have not made any isolations. Purchased B31 strain spirochetes (ATCC) grow in all the media. Since we have a low infection rate in the ticks in Missouri, it is possible that we have not had many infected ticks in our culturing attempts. This makes this type of endeavor very difficult and requires a great deal of work for success. However, the reward for success would be significant in learning more about the clinical problem

called Lyme disease. We must also remember that there are some pathogenic organisms that have never been successfully cultured.

OBSERVATION OF ARTHRITIS AND CARDITIS ON LYME DISEASE USING DIFFERENT AGES OF LABORATORY MICE INFECTED WITH VARIOUS ISOLATES OF *BORRELIA BURGDORFERI* BY TICK BITE AND NEEDLE TRANSMISSION

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The incidence rates of arthritis and carditis, and the antibody responses of different ages of C3H/He mice (6 wks and 12 mo of age) infected with various isolates of *Borrelia burgdorferi* (WI-210, isolated from *Ixodes dammini* from Wisconsin and CH-2246, isolated from *Ixodes persulcatus* from northeast China) were compared. Four groups (two young and two old) of C3H/He mice were infected by tick bite and two additional groups of old C3H/He mice were infected by needle inoculation (id $2x10^6$ spirochetes/mouse). Each group consisted of five mice. Tick infection rates of both isolates were greater than fifty percent and six to eight nymphs were allowed to feed on each mouse. Six healthy young and old mice served as negative controls.

Serology investigation: Blood specimens were collected at day 3, 5, 7, 19, 14, 21, 28, 42 and 56 post-inoculation from four groups of old C3H/He mice. Indirect Enzyme-linked Immunosorbent Assay was used to evaluate IgM and IgG antibody response of the mice. Low passage B31 whole cell sonicate was used as antigen. IgM and IgG responses of the two needle inoculation groups were much higher and reached peak levels earlier than tick bite groups (unpaired *t* test result: t=7.69 and P<0.001). Western blot analysis, with homologous antigen (CH-2246 and WI-210) and antibody were conducted. Antisera showed more and stronger reaction bands than when heterologous reagents were used. Analyses involving heterologous reagents, either WI-210 antigen reacted with CH-2246 antisera or CH-2246 antigen reacted with WI-210 antisera, showed fewer weaker bands. All homologous and heterologous reactions were weaker in mice exposed by tick bite than those obtained from mice exposed by needle inoculation.

Pathology results: All four groups of old C3H/he mice (12 mo of age) did not develop any arthritis and developed no more than sixty percent carditis. The two young C3H/He mice groups (6 wks of age) infected with CH-2246 antigen developed one hundred percent carditis and sixty percent arthritis while the group infected with WI-210 antigen developed sixty percent carditis and one hundred percent arthritis. However, larger numbers of mice are needed to verify whether the American strain causes more arthritis and the Chinese strain causes more carditis.

From this study, we conclude older C3H/He mice (greater than twelve months of age) are not suitable for modeling Lyme disease because they only developed mild carditis and did not develop arthritis. Young C3H/He mice (6 wks of age) showed different incidences of arthritis and carditis, depending on the strain of *B. burgdorferi* used to infect them. IgG and IgM responses are higher after needle inoculation of cultured spirochetes than after infection produced by tick bite, and immunoblots of four groups of sera (2 strains, tick and needle inoculation) differed significantly, depending on the antigen (WI-210 or CH-2246).

CHARACTERIZATION OF A *BORRELIA* ISOLATE FROM FLORIDA

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Recently, we identified spirochetemia temporally-associated with illness in two dogs from Florida. Clinical abnormalities included lethargy, fever, weight loss, pallor, anterior uveitis, lymphadenopathy, splenomegaly and conscious proprioceptive deficits. Laboratory abnormalities included anemia, thrombocytopenia, mild neutrophilia with a regenerative left shift, slightly high serum alkaline phosphatase, creatinine phosphokinase, lactate dehydrogenase, amylase and lipase activities, and rare granular urine casts. These laboratory abnormalities have not been reported in association with canine Lyme disease, and would be considered atypical for human Lyme disease.

Spirochetes cultured from one dog, grew abundantly in BSK-II culture media. The genus-specific monoclonal antibody H9724 bound to the organisms grown in vitro, indicating that the spirochete is a *Borrelia* species, but monoclonal antibody H5332 did not bind to the isolate, indicating that the organism is probably not *B. burgdorferi.* Polymerase chain reaction analysis, performed by E. K. Hofmeister, Johns Hopkins University, School of Hygiene and Public Health, using *B. burgdorferi*-specific osp A primers failed to amplify DNA from the cultured organism, again suggesting that the spirochete is not *B. burgdorferi.* Additional efforts to speciate the isolate are ongoing.

In both dogs, acute and convalescent immunofluorescence antibody (IFA) titers were higher to the Florida *Borrelia* isolate than to *B. burgdorferi.* Acute and convalescent sera did not contain antibodies to *Leptospira* serovars. Both dogs became clinically healthy following treatment with tetracycline hydrochloride.

A limited serosurvey of 99 dogs from the region identified 17 samples that were reactive by IFA to the Florida *Borrelia* isolate with reciprocal titers ranging from 64 to 512. Of these 17 samples, 4 were also reactive to *B. burgdorferi* antigen.

In a pilot experiment, we were able to demonstrate spirochetemia, on Wrights-Giemsa stained blood smears, in pre-weanling mice, rats and guinea pigs following intraperitoneal (mice) or intradermal (rats and guinea pigs) inoculation of the NCSU canine *Borrelia* isolate. Illness, characterized by fever, mild lethargy and severe hyperemia of the skin was observed in the guinea pigs. Transient lethargy and anorexia was observed in the rats. Two of 6 mice died during the 48 day observation period, and myocarditis was observed in one mouse.

From our preliminary efforts, we conclude that a member of the genus *Borrelia* causes spirochetemia in dogs in Florida and that the organism can induce serological cross-reactivity to *B. burgdorferi* antigen. The organism appears to infect a variety of rodent species and because it is a *Borrelia*, the spirochete is presumably tick-transmitted. The geographic distribution, mode of transmission, and pathogenicity of this organism require additional study.

EXPERIMENTAL TRANSMISSION OF SEVERAL GEORGIA ISOLATES OF *BORRELIA BURGDORFERI*

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Sapelo Island is located just off the coast of Georgia, south of St. Catherine's Island and north of Wolf Island. There are a variety of animals found on this eight mile long island including raccoons, deer, cotton mice, cotton rats, several species of lizards, and opossums. Adult *Amblyomma americanum* and *Ixodes scapularis* are abundant on Sapelo Island. Isolations of *B. burgdorferi* were made from cotton mice, cotton rats, and a black-legged tick. An isolate (SI-1) from a cotton mouse (*Peromyscus gossypinus*) and one (SI-3) from a cotton rat (*Sigmodon hispidus*) were used in transmission studies with *I. scapularis* and *A. americanum*. Both SI-1 and SI-3 reacted positively to two Osp A (H3TS, H5332), two Osp B (H5TS, H6831), and the genus specific H9724 monoclonal antibodies. They did not react to the *B. hermsii* 9826 nor to *B. coriaceae* F6F3, F6B3 or F6B11 monoclonal antibodies. Both isolates were positive for the flagellin and Rosa's chromosomal gene sequences for *B. burgdorferi* using PCR; SI-1 was positive and SI-3 was negative for the Osp A gene sequences. SDS-PAGE analysis revealed that SI-3 was similar to SI-1 except a single Osp region band and a low molecular weight protein band at 27 kilodaltons were present in SI-3.

Approximately 10^7 of each isolate (SI-1 and SI-3) was injected into hamsters, white mice, or cotton mice. Several cotton mice were inoculated with the SI-3 isolate to determine if an isolate from a cotton rat could infect cotton mice as well as be transmitted to other hosts. After 8 weeks, *I. scapularis* or *A. americanum* larvae were placed on inoculated hosts. Nymphs resulting from the fed larvae of both species were placed on naive white mice. The naive white mice were cultured 8 weeks after nymphal feedings. Ear clips, bladders, kidneys, hearts, and spleens were maintained in BSK-II media at 34 °C for 8 weeks. Fed larvae, unfed nymphs, and adults were also assayed by culturing in BSK-II.

All organs from hamsters (n=4) inoculated with the SI-1 isolate were culture positive (spirochetes were reisolated from these hosts). All organs from white mice (n=12) fed on by *I. scapularis* nymphs from SI-1 feedings were culture positive, however, all organs from white mice fed on by *A. americanum* were culture negative. The control hamster inoculated with SH2-82 isolate and naive white mice fed on by *I. scapularis* nymphs resulting from larval SH2-82 feedings were culture positive. Additionally, all organs from white mice (n=3) inoculated with the SI-3 isolate were culture positive as were all naive white mice (n=3) that were fed on by infected *I. scapularis* nymphs. All naive white mice fed on by nymphal *A. americanum* were culture negative. All organs from two of the four inoculated cotton mice were culture positive and three of the naive white mice fed on by *I. scapularis* infected by feeding on inoculated cotton mice were culture positive. Fifty-three and twenty-four percent of the *I. scapularis* unfed nymphs from the SI-1 and SI-3 transmission studies, respectively, were culture positive.

In summary, *I. scapularis* can transmit the SI-1 and SI-3 isolates to white laboratory mice (*Mus musculus*) and cotton mice (*P. gossypinus*). *A. americanum* does not appear to be able to transmit either isolate. The infectivity of hamsters and white mice with SI-1 and SI-3 appears to be similar. We are continuing transmission studies with *I. scapularis* and *A. americanum* with these isolates and several others from Georgia and Florida.

BORRELIA BURGDORFERI IN EASTERN VIRGINIA

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The infection of two tick species and various rodent hosts with *Borrelia burgdorferi* present an emerging picture of the Lyme disease spirochete cycle in eastern Virginia. During the past two years, a total of 16 isolations have been made from feral house mice, white-footed mice, least shrews, and rice rats. The coastal areas have been the source of most of the infected mammals. The presence of infected *Ixodes dammini ticks* and white-footed mice demonstrate that this area of Virginia has an established *B. burgdorferi* cycle similar to that reported in the northeastern United States. The role of the other hosts in maintaining the spirochete in this area has not been determined. The infection rates along the coast were determined by IFA on ticks and cultures of host tissues and serology. *I. dammini* and *Dermacentor variablis* ticks, least shrews, rice rats, feral house mice, and white-footed mice had infection rates of 7.7, 0.3, 3.6, 5.2, 2.9 and 4.2% respectively in this locality. Serologically, infection rates for feral house mice and white-footed mice were 47 and 64% respectively.

The infection rates on the mainland, York County, are lower compared to coastal Virginia. There have been two isolations from white-footed mice to date representing an infection rate of 1.9%. Serology from white-footed mice indicated that 30% were infected. Other hosts from the area have not produced any isolates and serology has not been completed. Of the approximately 400 *I. dammini* ticks collected in York County, two were infected with spirochetes. Thus spirochete infection of the common host and vector, including human cases of Lyme disease from the area, indicate the presence of an established *B. burgdorferi* cycle.

Xenodiagnosis has established the infectivity of cultured Virginia isolates. Needle inoculation of isolates 384, 395, and 396 infected laboratory mice whereas isolate 394 was not infectious. Two of the isolates, 384 and 396, also infected *I. dammini* larva which fed on inoculated laboratory mice. These experiments, involving 4 of the 16 Virginia isolates, demonstrate two key points about the Lyme disease cycle in coastal Virginia; *viz.*, Virginia isolates can infect mice and can be transmitted to *I. dammini* ticks. What remains to be determined is the ability of infected ticks to transmit infection.

Several additional findings may influence the coastal *B. burgdorferi* cycle and exposure to Lyme disease spirochetes. (1) Three new host species were found to be infected to coastal Virginia, feral house mice, least shrews, and rice rats. The effect that these hosts have as reservoirs was not determined because of the difficulties in keeping these animals alive in captivity. (2) One of the isolates, 394, was not infectious in xenodiagnosis experiments. The successful isolation stands as evidence that the organism had the ability to infect mice. Culture conditions may have contributed to the loss of infectivity, or this isolate may be a less infectious strain of *B. burgdorferi*. (3) Spirochetes were detected by IFA in another tick species, *Dermacentor variablis*. The ability of this tick species to transmit infection to be various hosts remains to be addressed. (4) Related to the issue of infectivity, it was observed that spirochete cultures were becoming non-infectious and simultaneously there was a major shift in the location of P39 from the membrane to the cytosol. How the change in P39 might be related to infectivity and the role this plays with Virginia isolates is under investigation. These observation reveal the complexity of the interactions of the Lyme disease spirochete cycle which ultimately will determine the risk of human exposure to *B. burgdorferi* in eastern Virginia.

THE EPIZOOTIOLOGY OF LYME BORRELIOSIS IN TEXAS

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Between January, 1990 and December, 1992, 763 possible cases of Lyme borreliosis were reported to the Texas Department of Health (TDH). Of these, 214 met the current definition for a confirmed case. The majority of patients lived in the north central portion of the state, though sporadic cases were identified throughout Texas. Sixty-eight (32%) of all confirmed patients experienced at least one skin lesion diagnosed by a physician as erythema migrans. Most cases of erythema migrans had onset between April and July. Seventy-five (35%) patients recalled an attached tick prior to onset of symptoms and 14 (7%) remembered a flea bite. In Texas, *Borrelia burgdorferi* has been isolated from *Amblyomma americanum*, *A. maculatum*, and *Ixodes scapularis* ticks as well as from the cat flea, *Ctenocephalides felis*.

During this same time period, ticks from eight Texas parks were collected and analyzed to determine the prevalence of spirochete-infected ticks. Spirochetes were detected in 1.03% of 5,141 *A. americanum* adults examined, a species Texas residents often encounter. No spirochetes were observed in the other tick species tested.

The TDH has been testing serum from dogs (the only non-human animal species from which the Lyme disease spirochete has been isolated in Texas) and cats suspected of being infected with *B. burgdorferi*. Signs in 194 dogs included lameness, fever, lethargy, anorexia, and neurologic abnormalities. Forty (20.6%) of these dogs were seropositive with titers of ≥ 1:256. In a separate study, 173 vaccinated and non-vaccinated dogs, without signs of infection, were tested. Seropositivity rates were 6.1% in the non-vaccinated group and 100% in the vaccinated group. Only 14 sera from clinically ill cats have been examined. Of these, 2 had antibody titers of 1:128, and one had a titer of 1:512. Signs given for these 3 cats included lameness, fever, weight loss, and lethargy. A serologic survey of 319 felines with no signs of infection resulted in approximately 16% with antibody titers of 1:128 or higher; 10% had titers of ≥ 1:256.

BREAK-OUT GROUP REPORTS

VECTOR & RESERVOIR COMPETENCE

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The discussion leader offered several questions to stimulate discussion. These included such issues as whether competency is an "either or" phenomenon?; if differing levels of infectivity or transmission efficiencies can influence competency; and whether there are physiological, biochemical and, perhaps, genetic determinants of competency.

The first issue discussed was PCR. Concern was expressed about the use of this technique to demonstrate infection. It was noted that PCR will detect fragments of DNA from dead organisms and that it is so sensitive that even a few remnants may be amplified. Consequently, if PCR can detect dead organisms as well as DNA from live spirochetes, does a PCR positive result prove that the tick or vertebrate host was infected? Similarly levels of infection in ticks or tick hosts may not be readily distinguished. This is important to understand vector reservoir competency. For example, PCR tests showed *B. burgdorferi* DNA in *Ixodes angustus*, but it was not clear that the ticks had become infected or that the spirochetes ingested had died without multiplying. Some scientists speculate that detectable DNA fragments may survive for 2 - 3 days after spirochetes that are acquired have died.

An interesting discovery concerning genetic markers and their relation to infectivity was discussed. In mosquitoes, scientists have reported differences in as little as one gene marker or even a single inversion that was correlated with the presence of infective transmissions. Dr. J. Oliver described work done in Switzerland (University of Neuchatel) where the SDS-Page profiles of tick extracts observed before the ticks became infected were changed after infection. Certain bands which were not expressed prior to tick infection were expressed after the ticks became infected. However, Dr. Piesman questioned the significance of the Swiss work, noting that the ticks were deliberately infected by capillary transmission with millions of spirochetes. He suggested that the pathogen burden was unusually heavy and not representative of natural infection. Such heavy burdens of foreign organisms may have affected the tick's biochemical activity and led to the altered profiles.

Genetic variability may be related to the variety of vectors and reservoir hosts. Dr. J. Piesman theorized that in northeastern United States, where Lyme borreliosis is highly focused in one efficient vector (*Ixodes scapularis*) and one primary small mammal reservoir (*Peromyscus leucopus*), there is little genetic variability. In the southeastern U. S., where the zoonosis is potentially dispersed over a wider variety of vectors and reservoir hosts, there is greater opportunity for genetic diversity.

Another issue that was discussed was whether the magnitude of the pathogen population achieved in a vector tick and, consequently, its ability to transmit infection, was related to pathogen specificity (i.e., strain). The JD-1 strain of *B. burgdorferi* was transmitted by *Ixodes dentatus* but not as readily as other strains, e.g., B-31. *I. scapularis* is said to be at least three times more efficient that *I. pacificus* in acquiring *B. burgdorferi* infection, even when the California strains are used.

Considerable interest was directed to the question of the (presumed) *B. burgdorferi* isolates from the lone star tick, *Amblyomma americanum.* Several laboratories have made such isolates and noted that they seem difficult to culture. In addition, transmission by lone star ticks of these isolates has not been reported. However, Dr. Piesman noted that the cultures of spirochete isolates from lone star ticks that he had received had already gone through 6 or 7 passages and may have lost infectivity. He indicated that attempts were now in progress to compare the isolates with other spirochete strains by profiling their DNA.

Finally, the role of lizards in the ecology of Lyme borreliosis was discussed. Lizards have been thought to be incompetent as reservoirs of *B. burgdorferi* infection. However, recent work at N. C. State University has shown that some lizards can be infected and can infect ticks that feed on them. However, as Dr. J. Levine noted, this laboratory study was done with very large numbers of infected ticks feeding frequently, i.e., exposed to frequent high doses of infectious feedings, a scenario unlikely to occur very often in nature. The significance of this very interesting finding is unknown.

LYME DISEASE SURVEILLANCE AND LANDSCAPE ECOLOGY

Durland Fish - discussion leader

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The topic of the session was changed from landscape ecology to include surveillance because it was felt that surveillance methods needed to be discussed prior to a discussion of landscape ecology of Lyme disease in the Southeast. The identification of endemic areas is less of a problem in the Northeast where Lyme disease is more prevalent both in nature and in humans. Application of landscape ecology methods to the Southeast are of limited value without more information on the distribution and intensity of transmission risk in nature.

The objective of surveillance for Lyme disease should be to measure human risk of infection. The methods used to measure human risk at a given point in time and space should be repeatable in order to detect changes in risk (i.e. spreading or emerging risk). Components of risk for Lyme disease include human biting rate by vector ticks, vector tick density, and pathogen prevalence in vector ticks.

Human biting rate for vector ticks is difficult to quantify. Identification data from tick specimens submitted by the public or veterinarians to health agencies or academic institutions can indicate the relative prevalence of host-seeking species in the environment for specific locations. However, these data have limitations in that such passive surveys may not accurately reflect human biting rate. Canine data may include species or stages that do not feed readily upon humans, and immature stages of *Ixodes scapularis* can feed to repletion on humans without notice.

Data are needed to establish relationships between vector density and human biting rate. Data from controlled human use of tick-infected areas, such as military installations or scout camps, could be useful in establishing the relationship between vector density and human contact. But, the assumption that all ticks acquired on clothing or skin will ultimately feed upon humans has not been validated.

Vector tick density estimates can be obtained by standard tick sampling procedures, such as dragging or flagging for host seeking specimens and examining common host species. Carbon-dioxide baited traps are not usually effective for *I. scapularis,* but periodic examinations of artificial mouse "houses" have been found to be productive in the Northeast. All such measures of relative density need to be calibrated with estimates of absolute population density from mark-release-recapture experiments in order to provide accurate estimates of actual density.

Knowledge of the seasonality of life stages is an essential prerequisite to the estimation of vector population density. Life-stage phenology varies geographically and density estimates for each stage should be synchronous with its peak activity period. Adult *I. scapularis* probably is more easily sampled than immature stages throughout most of its range. This may be true not only for the host-seeking portion of the population, but also for the feeding population attached to hosts. White-tailed deer appear to be the most common host for adults throughout the range of *I. scapularis*. Hunter-killed deer are commonly available in most southeastern states when adult ticks are active. Infestation rates of *I. scapularis* on white-tailed deer from hunter-killed samples therefore may be the simplest universal measure of vector abundance. A minimum of 50 samples from an area should be sufficient.

The infection rate of *Borrelia burgdorferi* in *I. scapularis* is influenced by the method of specimen collection, the life stage of the sample, and the pathogen detection method. Ticks removed from hosts may not accurately reflect the infection rate of the host-seeking population which poses the risk for humans. Uninfected ticks may acquire infection from hosts during feeding causing elevated infection rates. Also, blood may interfere with pathogen assays resulting in false negative results. Therefore, only host-seeking ticks should be used for infection rate determinations. Infection rates in *I. scapularis* that are stage specific in the Northeast are likely to be so throughout the range. Adults have higher infection rates than immatures and therefore make better candidates for infection rate determinations.

Spirochete determination methods include darkfield microscopy, fluorescent antibody, antigen-capture ELISA, polymerase chain reaction, and culture. The accuracy of infection rates as determined by each of these methods is unknown. In the absence of a gold standard for determining *B. burgdorferi* infection rates in ticks, darkfield microscopy should provide adequate results for surveillance purposes and it is the simplest method available.

Serology of wildlife and domestic animals is often used to measure the distribution and prevalence of *B. burgdorferi*-specific antibodies in non-human populations. The value of wildlife serology is questionable in view of the likelihood of cross-reactivity and the problems involved with both false negatives and false positives in human serology. Canine serology appears to correlate well with vector density in the Northeast, but studies in the Southeast have reported seroprevalence rates at or below that of control samples.

The application of reliable and standardized surveillance methods for Lyme disease in the Southeast, as well as elsewhere, is likely to reveal spatial patterns of endemic foci attributable to landscape features. The analysis of landscape features in relation to risk attributes discussed in this session will provide a framework for studying the landscape ecology of Lyme disease in the Southeast.

CASE IDENTIFICATION AND DISTRIBUTION

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Surveillance for human Lyme disease (LD) in the southeast differs from the northeast because of the relatively small number of cases in the south. This makes the impact of inaccuracies in diagnosis or reporting potentially more dramatic in the south. When targeting ecological field studies based on the geographic distribution of human cases, there is a risk that the data on human cases may not be reliable.

The problems associated with human LD case identification include: 1) the lack of a "gold standard" for diagnosis (depending on physicians to accurately evaluate clinical signs, especially in early LD when serology is likely to be negative, and on non standardized serologic tests for late LD); 2) under diagnosing and under reporting; and 3) over diagnosing and over reporting. The latter is more likely with LD than many other reportable diseases due to the media attention and the existence of "LD activists." When large numbers of patients who are unlikely to have the disease are tested, the likelihood of a false positive result is increased.

The following were suggestions to aid in improving case identification and providing more reliable distribution data:

1. Encourage submission of skin biopsies from appropriate cases to provide evidence that *Borrelia burgdorferi* infects southeastern patients.

2. Develop a better isolation media/system that can more readily identify different strains as well as maintain infectiousness longer.

3. Provide educational programs for physicians to help them identify cases more accurately and improve reporting.

4. Evaluate the utility of submitting acute and convalescent serum samples from patients with erythema migrans to document the presence of LD in the Southeast.

5. Provide more resources for conducting active surveillance.

6. Conduct case control or other special studies on captured populations that can be better documented.

7. Encourage the publication of reports on southeastern cases in peer reviewed scientific journals.

MODELING LYME DISEASE

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Computer modeling provides an additional tool for the study of tick population dynamics, disease transmission, and control technologies. At the Workshop, a preliminary model for Lyme disease was displayed and discussed by a group of six participants. The displays consisted of color text and graphic

output from simulations of vector populations and Lyme disease transmission in New York, Florida, and Minnesota. The group discussed the need for additional research to improve the accuracy and validity of the model. The following areas were identified.

(1) **Density of *Ixodes scapularis*:** No data are available in the literature on the density of *I. scapularis*. Mark-release-recapture experiments are needed in a variety of habitats and locations in the eastern U. S. to determine tick densities. These studies are important because of the relationship between vector density and level of disease transmission. Density studies are also needed to determine vector population trends.

(2) **Development rates for *Ixodes scapularis* from different geographic regions:** Preliminary model development and calibration suggest differences in development rates for *I. scapularis* from different geographic regions. Simulated tick populations grown with Wisconsin and Minnesota weather files required development rates about 15% greater than populations in the East. Thus, comparative laboratory studies are needed with strains of ticks recently collected from the Great Lakes area and the East. Investigations with existing laboratory colonies adapted to constant laboratory temperatures may not reflect differences that exist in natural populations.

(3) **Feeding success of *Ixodes scapularis* on hosts:** A density-dependent factor regulating population growth is the feeding success on various host animals. Very little information has been published in the literature. Experiments with successive tick feedings on previously naive host animals are needed to estimate the potential of various types of hosts to develop tick resistance. Data on key hosts such as white-tailed deer and white-footed mice are especially needed.

(4) **Relationships between tick activity and temperature or season (daylength):** Preliminary modeling experience suggests variation in tick populations from different geographic regions concerning host-finding activity. Although little information is available in the literature, seasonal activity patterns for *I. scapularis* in different geographic regions suggest some variation in response to ambient temperature. Although relationships between host-finding activity and daylength are used in the model for immature stages, no supporting data are available in the literature. Thus, studies on tick activity, as related to temperature and daylength, are needed to either support or modify the existing model on *I. scapularis* and transmission of the Lyme disease agent.

CLADISTIC ANALYSIS OF SELECTED TICK TAXA

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A phylogenetic analysis is carried out to re-examine relationships at the generic level among the hard ticks, family Ixodidae, with the ultimate goal of generating a well corroborated phylogeny that can be used in testing evolutionary hypotheses.

The analysis was based on 71 morphological and cytogenetic characters, 20 of which are multi-state. All of these characters are intrinsic to ticks (host associations are considered extrinsic), have distinct states (no continuous variation), and show relatively low within-taxon variability. Taxon selection was aimed at including the largest possible diversity. Wherever possible this was achieved by including entire genera (for the smaller genera) or subgenera (for the larger genera) as end taxa. A well supported subgeneric classification for the large genera *Amblyomma* and *Rhipicephalus* is presently not available, and these genera were represented by a number of examplar species. A total of 46 ingroup taxa was considered.

Character polarity was established by outgroup comparison using the tick families Nuttalliellidae and Argasidae, and the Parasitiform suborders Holothyrida and Mesostigmata. All analyses were conducted using the computer algorithm PAUP, using heuristic search procedures with 10 repetitions.

Monophyly of the family Ixodidae was supported by at least 7 characters, corroborating traditional views of the classification of the group. Within the subfamily Amblyomminae (Metastriata) we found good support for monophyly of the genera *Haemaphysalis* and *Hyalomma*, but not for *Aponomma*, *Amblyomma*, *Dermacentor*, and *Rhipicephalus* (although some of the equally most parsimonious trees featured a monophyletic *Rhipicephalus*). Restraints analyses forcing monophyly of those genera required, respectively, 4, 5, 3, and 0 additional characters changes or "steps". Not all groupings emerging from a strict consensus tree of all equally most parsimonious trees for the Amblyomminae are equally well supported, a problem that should be considered when using such trees in subsequent evolutionary studies. To quantify relative support, we calculated the minimum number of additional steps required to make a given lineage not monophyletic (the decay index = DI) for all nodes in the consensus tree. Monophyly of the subfamily Amblyomminae is well supported (DI = 9), as is monophyly of the groupings consisting of *Cosmiomma*, *Dermacentor*, *Rhipicentor*, *Anomalohimalaya*, *Nosomma*, *Hyalomma*, *Rhipicephalus*, *Boophilus*, and *Margaropus* (the Rhipicephalinae and Hyalomminae sensu Hoogstraal) (DI = 6), and of *Nosomma* and *Hyalomma* (DI = 5).

The subfamily Ixodinae (Prostriata) contains only a single genus, *Ixodes*, with many subgenera. We followed the subgeneric classification proposed by Clifford et al. (1973, Ann. Entomol. Soc. Am. 66 (3): 489-500) with the addition of *Ixodiopsis* (split off from *Pholeoixodes*) and the monotypic subgenus *Coxixodes*. Some of these subgenera may not be monophyletic. The Australian members of the subgenus *Exopalpiger* appear quite distantly related to the S. American and Palearctic species in that subgenus. Similarly, the S. American marsupial ticks *I. luciae* and *I. loricatus* did not group with the remaining members of the subgenus *Ixodes*. Support for most lineages in the consensus tree (based on decay indices) was weak, but we found fairly good support for the monophyly of the genus *Ixodes* (DI = 4) and for a lineage comprising most species, with the exception of the endemic Australian subgenera *Endopalpiger*, *Sternalixodes*, and *Coxixodes*, and the Australian members of *Exopalpiger* (DI = 3).

Overall the results of the systematic analysis indicate some well supported groupings, although support for many other lineages is weak. The monophyly of these lineages should be tested using additional characters. Despite the uncertainty of some of the systematic results, they can be used in preliminary analyses of tick evolution. In one striking pattern, it appears that the basal lineages in both Ixodinae and Amblyomminae are nearly all exclusively Australian in range. The only exception, a lineage of the "typical" *Aponomma*, has some Australian members. This pattern, should it withstand further testing, strongly suggests that the family Ixodidae originated in Australia.

MORPHOMETRICS OF GEOGRAPHIC POPULATIONS OF *IXODES SCAPULARIS* SAY, 1821 (ACARI: IXODIDAE)

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Since 1979 northern populations of the black-legged tick, *Ixodes scapularis*, from MN east through southern Canada and New England and south through IL and MD, were considered a separate species, *I. dammini*. Justification for describing *I. dammini* was based largely on morphological differences in nymphal auriculae (larger and arising flush with the external basis in northern populations versus smaller and arising more mesad in southern populations) and internal spurs on coxae I of adults (shorter and anteriorly broader in northern populations) (Spielman, A. et al. 1979. J. Med. Entomol. 15: 218-234). The

possible influence of geographic variation on these quantitative differences was not reported. *I. dammini* was recently designated a junior subjective synonym of *I. scapularis;* assortative mating indicated lack of a reproductive barrier between populations from MA and GA; the degree of variation in isozymes and chromosome c-banding were also indicative of conspecificity. In addition, size-free (sheared) discriminant analysis of 23 nymphal, 17 female and 25 male morphological characters, including those used to separate southern from northern forms, showed differences between populations from GA or NC and MD or MA. Differences were strongly correlated with a general 'size' factor and are probably ontogenetically or environmentally labile. They are also suggestive of variation among geographic populations of a single species (Oliver, J. H. et al. 1993. J. Med. Entomol. 30: 54-63). Comparisons of DNA sequences also support conspecificity (Wesson, D. M. et al. 1993. Proc. Nat. Acad. Sci. 90). As a synonym, *I. dammini* joins five other names in that category, including *I. ozarkus* Cooley & Kohls, 1944; *I. ricinus* var. *scapularis* Nuttall & Warburton, 1911; *I. pratti* Banks, 1908 (pro parte); *I. reduvius* Neumann, 1899 (pro parte); and *I. fuscous* Say, 1821 (Keirans, J. E. & C. M. Clifford. 1978. J. Med. Entomol. Suppl. No. 2: 1-149). The pattern of intraspecific morphological variation is suggestive of a latitudinal cline and possibly a longitudinal cline. This work presents preliminary results of a multivariate morphometric study of geographic populations of *Ixodes scapularis*.

Ticks used in this study were laboratory reared to minimize short-term environmental effects. They originated from Worcester Co., MD; Dare Co., NC; Chisago Co., MN; Pulaski Co., MO; Bulloch Co., GA; Great Is., MA; F_1 progeny from MA X GA reciprocal crosses. Measurements were made from cleared, microslide-mounted larvae (34 characters), nymphs (24 characters), females (17 characters), and males (25 characters). Discriminant analysis revealed the greatest differences among groups of nymphs; the first function, which accounted for ca. 55% of the between-group variation, separated northern (MN, MA, MD) and southern (GA, MO, NC) groups; both crosses were intermediate. The second function, which accounted for ca. 32% of the between-group variation, separated the western (MN, MO) and eastern populations. Scatterplots representing ca. 87% of the total between-group variation suggest differences between western (MN, MO) and eastern (MA, MD, NC, GA) populations are as great or greater than those between northern (MN, MA, MD) and southern (MO, NC, GA) populations. Moreover, separation of north-south or east-west populations by each function suggests latitudinal (north-south) and longitudinal (east-west) clines. Scatterplots generated from size-free discriminant analysis showed that these differences were largely influenced by a general size factor (positive correlations with the first principal component). Univariate analyses revealed *I. scapularis* from Missouri are significantly smaller than all other groups ($P<0.01$) in relation to several characters of nymphs and adults, but are largest ($P<0.01$) in relation to scutal width. In contrast to studies of the rabbit tick, *Haemaphysalis leporispalustris* (Packard), (Thomas, P. A. 1968. Univ. Kan. Sci. Bull. 47:787-828), *I. scapularis* from northern populations (MN, MA, MD) are not significantly larger ($P\geq0.01$), but do have longer intercornua and interauriculae distances and relatively longer internal hypostome denticle files ($P<0.01$). Larvae and nymphs do not have higher palpal L:W ratios ($P\geq0.01$), and the internal spur of coxae I of adults is not broader ($P\geq0.01$) as reported by Spielman et al. (1979). Spurs are longer than those of adults from MO ($P<0.01$), shorter than those of adults from NC ($P<0.01$), and are not different from the other groups ($P\geq0.01$). Only three nymphal characters (left-right auriculae "tip" distance, left-right cornua "tip" distance, and hypostome internal denticle file length relative to hypostome length) appear to be positively correlated with latitude. Missouri *I. scapularis* are significantly different from all other groups in relation to 9 of 34 larval, 16 of 24 nymphal, 11 of 17 female, and 12 of 25 male characters ($P<0.01$). Each of the other populations are significantly different ($P<0.01$) from all other populations in relation to at least 1 or as many as 13 characters. Progeny of MA X GA crosses are morphologically intermediate, thus the morphological differences between geographic populations are probably genetically determined rather than environmentally influenced. Because MN and MO populations appear to be different from eastern populations, it is likely that the morphological and genetic variation among geographic populations of the black-legged tick are longitudinally as well as latitudinally clinal in nature.

RELATIONSHIPS AMONG SPECIES OF THE *IXODES RICINUS* COMPLEX ASSESSED BY THE PRESENCE AND ABUNDANCE OF HIGHLY REPEATED DNA

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Phylogenetic relationship among member species of the *Ixodes ricinus* complex are currently being assessed with morphological, behavioral, and molecular genetics data. Our objectives are to complement molecular genetics studies based on DNA sequencing by examining the distribution of highly repeated DNAs among member and outgroup species. By treating the presence and abundance of repeated DNA as multistate characters, our approach permits a traditional cladistic analysis rather than the phenetic or distance-based analyses characteristically applied to sequence data. Thus, we have subcloned and initiated the characterization of 18 putatively highly repeated DNAs from the genome of *Ixodes scapularis* (from Georgia). Characterization has entailed sequencing 250 bases from each end of the subcloned DNA using the dideoxy method on double-stranded plasmid DNA. Further characterization will involve Southern blotting and in situ hybridization to determine distribution within the genome and will involve additional DNA sequencing.

Highly repeated DNAs are a heterogeneous assemblage that includes simple sequences of only a few base pairs in length that occur in tandem, particularly in telomeric and centromeric constitutive heterochromatin, and more complex sequences of up to 1000 bases scattered in heterochromatin and euchromatin. Some may represent huge families of transposable elements with or without the capacity for autonomous transposition. The copy number of repeated elements can vary tremendously between species as a consequence of non-Mendelian mechanisms such as unequal exchange and gene conversion. Highly repeated DNAs appear to be under weak if any selective constrain based on sequence of non-Mendelian mechanisms such as unequal exchange and gene conversion. Highly repeated DNAs appear to be under weak if any selective constrain based on sequence and copy number. Consequently, they can provide information on relationships within recently evolved assemblages.

Eco **RI** digested genomic DNA of *I. scapularis* was subcloned into the plasmid *pBluescript SK+*. Dot blots of 480 subclones were hybridized with ^{32}P-labeled total genomic DNA. Intensity of hybridization was assessed by autoradiography, revealing putatively highly repeated DNAs. Eighteen subclones were then dotted and probed, in 4 replicated, with total genomic DNA of the complex species, *I. affinis, I. pacificus, I. persulcatus, I. ricinus,* and *I. scapularis,* and of outgroup species, *I. woodi, Amblyomma variegatum,* and *Dermacentor nitens. A. variegatum* genomic DNA failed to hybridize to any subclone dots. All other species' DNA hybridized to at least some subclones with the intensity of hybridization varying greatly by species and by subclone with a species.

Sequencing and subsequent analysis using PC Gene programs revealed the absence of sequence similarity among the 18 subclones. Tandem repetition was rare within subclones although simple palindromic sequences were found to occur in tandem arrays of up to 7. Most subcloned DNA possessed several groups of degenerate repeats of 5 to 35 base pairs in length. Repeated sequences were not enriched with A/T.

A phenetic **Neighbor-Joining** analysis based only on the absence or presence of subclones in *I. ricinus* complex species placed *I. scapularis* close to *I. ricinus*, with *I. pacificus* closer to these than was *I. persulcatus* from the *I. scapularis/I. ricinus* cluster. Cladistic analysis corroborated the **NJ** analysis with regard to *I. ricinus* complex species. Among outgroups, *I. woodi* was placed intermediate between *I. affinis* and the non-*Ixodes* species.

The relationships revealed are not consistent with current biogeography. *I. persulcatus* does not group closely with *I. pacificus* in spite of the recent Bering Sea land bridge that united their respective ranges. Furthermore, *I. ricinus* does group closely with *I. scapularis* although the Atlantic Ocean has long been a dispersal barrier between their respective ranges.

A PHYLOGENY OF HARD AND SOFT TICK TAXA BASED ON MITOCHONDRIAL 16S rDNA SEQUENCES

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Ticks are mesostigmatid mites that are obligate hematophagous ectoparasites on reptiles, birds and mammals. Ticks are placed in three families: Argasidae (soft ticks), Ixodidae (hard ticks) and Nuttalliellidae (a monotypic family sharing characters of both Argasidae and Ixodidae). Most ticks are host specific and it has been suggested that their evolution and life histories are intimately associated with host evolution. A phylogeny for ticks has been described based on morphological characters, life histories and host associations. Towards testing the existing phylogeny, we sequenced approximately 460 bp from the 3' end of the mitochondrial 16S rDNA gene in 35 hard and soft tick taxa and a closely related hematophagous mesostigmatid mite was used as an outgroup. Phylogenies were derived using both distance and parsimony methods and the consistency of nodes was tested using bootstrap analysis. The derived phylogeny at or below subfamilies largely supports the traditional phylogeny with two exceptions in Ixodidae. Members of the primitive Amblyomminae did not form a monophyletic group and members of Hyalomminae grouped within the Rhipicephalinae. The derived phylogeny above subfamilies provided strong evidence that the subfamily Argasinae (Argasidae) forms a basal and monophyletic group with the Ixodidae suggesting that hard ticks originated from an *Argas*-like ancestor. Because most *Argas* species are obligate bird ectoparasites, this result may suggest that hard ticks did not evolve until the late Cretaceous, much more recently than originally suggested.

THE TAXONOMIC STATUS OF *IXODES DAMMINI*

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Before the middle of the 20th Century, intense infestations of *Ixodes persulcatus*-like ticks in North America were restricted to the Elizabeth Islands, in Massachusetts, where a relict herd of deer remained extant. Although occasional *I. scapularis* deer ticks were recorded in various sites in New York and New England in the early 20th century, the only known intense infestation in the 1920s was on Naushon Island. Neighboring Martha's Vineyard and Nantucket Islands were intensely infested by a mouse tick, *I. muris* but not deer ticks. Deer had become virtually extinct elsewhere in the northeastern U. S. by the end of the 19th Century. The herd began to increase with the onset of the 20th Century. This rate of increase appears to have accelerated during the last several decades. The first intense mainland deer tick infestation was recognized in Rhode Island in 1960. Similar but structurally distinct ticks, designated *Ixodes scapularis*,

infested sites in the southeastern quadrant of the U. S., at least 400 km distant. Because these populations were geographically isolated and had diverged morphologically, the northern ticks were designated as a separate species, *I. dammini*. The first mainland infestation of this tick was recognized in 1960; the first American case of human Lyme disease in 1963 and babesiosis in 1969. The range of this tick subsequently expanded and now includes much of the eastern and north-central U. S. Although laboratory colonies isolated from Massachusetts and from Georgia in the 1970s could not interbreed, one pair of such colonies, established in the 1980s, were said to interbreed freely. We conclude that *I. dammini* differs from *I. scapularis*, but that its range has merged into that of its southern sibling such that laboratory isolates may be contaminated. *I. dammini* is a valid specific designator. Analysis of 16S mitochondrial DNA sequences indicated that *I. dammini* populations sampled from diverse northern sites differ by no more than a few base-pair, and that at least 17 base-pairs distinguish these populations from those of *I. scapularis*. Other members of the *I. persulcatus* complex of ticks are more distinct. These considerations require the retention of *I. dammini* as a specific designator.