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Editorial content: Topics relating to understanding disease mechanisms and the application of better diagnostic techniques and treatment strategies for all individuals suffering from spirochetal and tick-borne diseases. Letters to the Editor in the form of correspondence related to material published in the Journal or some aspects of spirochetal and tick-borne diseases may be submitted. Such letters, if related to work previously published in the journal, will be referred to the author of the original work for a response.
Editorial

Another Look at the Potential Role of Amblyomma americanum in the Transmission of Tick-borne Disease

Terry L. Schulze, PhD and Edward M. Bosler, PhD

Until the mid-1970s, tick-borne diseases affecting humans in the Northeast and mid-Atlantic states remained more of a medical curiosity than a public health threat. Prior to that time, the only tick-borne disease of any significance in this region was Rocky Mountain spotted fever. The principal vectors of the etiological agent, Rickettsia rickettsii, are the American dog tick, Dermacentor variabilis Say; and the lone star tick, Amblyomma americanum L. In most of the Northeast, A. americanum is considered as a secondary vector, primarily because of its limited geographical distribution. A. americanum is known mainly for its economic importance as a serious pest of humans, livestock, and wildlife.

The relative insignificance of tick-borne diseases to the entire spectrum of public health changed dramatically with the description of Lyme disease in Connecticut in 1975. Based on epidemiological evidence, the black-legged or deer tick, Ixodes scapularis Say (formerly I. dammini Spielman, Clifford, Piesman, and Corwin) was implicated as the vector of the as yet unidentified etiological agent. Early investigations centered on I. scapularis, and in 1982, the spirochete Borrelia burgdorferi was found in this tick species collected from eastern Long Island, NY. B. burgdorferi subsequently was isolated from I. scapularis from the northern Midwest and I. pacificus Cooley & Kohls in the West. In less than a decade, a complete understanding of the epidemiology of Lyme disease seemed at hand.

Early studies of Lyme disease foci in New Jersey showed that A. americanum temporally and spatially coexisted with I. scapularis. New Jersey and parts of southeastern New York, however, mark the northern extent of significant populations of A. americanum, so this tick was not considered a potential vector of Lyme disease elsewhere in the Northeast. The vector potential of the lone star tick first became evident in 1982, when an A. americanum female was removed from the site where erythema migrans (EM) later developed in an 87-year-old male from Medford, NJ. This case predated the discovery of B. burgdorferi. In 1983, a second case of Lyme disease associated with A. americanum was reported when a tick was removed from the site at which EM developed in a 37-year-old female from Barnegat, NJ. Spirochetes subsequently were identified in A. americanum adults and nymphs collected from the place of residence of the second case and elsewhere in New Jersey. Although garnering some early interest in the scientific community, the potential importance of A. americanum in Lyme disease transmission was dismissed when early efforts to culture spirochetes from this species were unsuccessful.

In the late 1980s, physicians from Georgia and Missouri began reporting an illness clinically indistinguishable from Lyme disease, but subsequent investigations failed to implicate B. burgdorferi. Although both I. scapularis and B. burgdorferi were present in this region, the most commonly reported tick exposure for patients was to A. americanum. Renewed interest in A. americanum spurred new investigations into the potential importance of this tick in Lyme disease transmission. Other than the earlier reports from New Jersey, spirochetes were found in A. americanum collected from Alabama, Indiana, Iowa, Missouri, North Carolina, Oklahoma, and Texas. Unsuccessful attempts to cultivate these spirochetes and the inability of A. americanum to transmit B. burgdorferi in the laboratory seemed irreconcilable. Recently, through the use of DNA sequencing, it has been postulated that this noncultivable spirochete retrieved from A. americanum is a new Borrelia sp., B. lonestari, which may be responsible for a new Lyme disease-like illness. This issue is far from resolved.

As the role of A. americanum in the transmission of Lyme disease or a new Lyme disease-like illness is being revisited, researchers will need to address the emergence of two new rickettsial diseases simultaneously, human mono-
cytic ehrlichiosis (HME) and human granulocytic ehrlichiosis (HGE), as serious potential threats to human health. *Ehrlichia chaffeensis*, the etiological agent of HME, is known to be transmitted by *A. americanum*. More than 400 cases of HME have been reported in the United States, including nine cases from New Jersey in 1995 (T.L.S., unpublished data). Approximately 170 cases of HGE, caused by an as-yet-unnamed *Ehrlichia* sp., have been reported since 1994, primarily from upper midwestern and northeastern states. Although *I. scapularis* is purported to be the vector of this new *Ehrlichia* sp., cases to date have been reported from outside the geographical range of *A. americanum*, so that the vector potential of this tick regarding HGE remains unclear. Interestingly, the first known outbreak of human ehrlichiosis in New Jersey occurred more than a decade ago in 1985, in July, when both *I. scapularis* and *A. americanum* are active.

Although the emergence of the *Ehrlichiae* is strikingly similar to that of Lyme disease nearly 2 decades earlier, a number of important differences exist. Owing to the sheer number of cases, Lyme disease has achieved a certain notoriety. As a result, public interest in other tick-borne diseases is considerable. In contrast to the emergence of Lyme disease, the recent recognition of *A. americanum* as another vector of Lyme disease or a new spirochetal illness and its role in the transmission of HME is likely to stimulate an already sensitized public to demand timely information from health agencies.

An important difference in the ability to provide information rapidly and develop strategies to deal with these emerging tick-borne disease issues is the current state of scientific knowledge. When Lyme disease was first described, little was known about the distribution, ecology, and control of *I. scapularis*. Well over a decade of research has been devoted to developing our understanding of this previously unimportant tick species. In contrast, because of its longstanding economic importance, the distribution, ecology, and control of *A. americanum* have been investigated more thoroughly, particularly in the southern United States. Much less is known about this tick along the northern extent of its geographical range. Compared to its economic importance, the role of *A. americanum* as a vector of human disease has received superficial attention. The distribution of *Borrelia* spp. and *Ehrlichia* spp. in *A. americanum* throughout its geographic range is poorly understood and should receive a high research priority.

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Vector Competence of Ixodid Ticks (Acari) for *Borrelia burgdorferi* as Determined With a Capillary-feeding Technique

Xiaohong Li, PhD and Robert S. Lane, PhD

**ABSTRACT**

Some factors that affect the vector competence of two ixodid ticks for *Borrelia burgdorferi* (Bb) were evaluated. The ticks included *Ixodes pacificus*, a proven competent vector and the primary bridge vector to humans in California, and *Dermacentor occidentalis*, an incompetent vector that rarely is found infected naturally with spirochetes. Adults of *I. pacificus* and *D. occidentalis* imbibed, on average, about $1.9 \times 10^6$ and $2.7 \times 10^5$ of Bb isolate CA4 in BSK-II medium, respectively, via a capillary-feeding technique. Spirochetes persisted in the midgut diverticula of both ticks for 5 months, but significantly more adult *D. occidentalis* died after infection. Despite the large spirochetal dose, none of the *D. occidentalis* (n=20) and only one of the *I. pacificus* (n=20) subsequently developed generalized tissue infections. When capillary-infected ticks were put on susceptible rabbits, only *I. pacificus* successfully transmitted spirochetes. Between 5 and 7 days after attachment to rabbits, the distribution of spirochetes within these ticks differed. Spirochetes in *I. pacificus* penetrated the midgut and disseminated to the salivary glands via the hemolymph in 30% to 40% of ticks, whereas dissemination did not occur in *D. occidentalis*, and spirochetes disappeared altogether from the midguts of 83% to 95% of ticks in different trials. These findings suggest that the vector incompetence of *D. occidentalis* is due to a midgut barrier that prevents spirochetal dissemination, to a borrelialadial factor associated with its feeding activities, or to both. We conclude that the capillary-feeding technique is a useful tool for studying the vector competence of ticks for Bb and for infecting ticks to be used in experimental transmission studies.

**Key words:** *Ixodes pacificus*, *Dermacentor occidentalis*, *Borrelia burgdorferi*

The Lyme disease spirochete, *Borrelia burgdorferi*, is maintained and distributed in endemic foci primarily by ticks in the genus *Ixodes*. In the western United States, *B. burgdorferi* is perpetuated in enzootic cycles involving ticks that do not bite humans (eg, *I. neotomae, I. spinipalpis*), woodrats, and other small mammalian hosts. *Ixodes pacificus*, a worrisome biter of humans and an efficient vector of *B burgdorferi*, also contributes to the chain of infection and serves as a bridge vector to people. In addition, several ticks in other genera occasionally have been found infected with spirochetes in this region of the country, including *Dermacentor occidentalis, Dermacentor variabilis*, and *Haemaphysalis leporispalustris*. Of these, *D. occidentalis* and *D. variabilis* attach to humans, but experimental studies have demonstrated that both ticks are incompetent vectors of Lyme disease spirochetes.

The vector competence of ticks for microbial agents is influenced by various intrinsic and extrinsic factors. Although specific biologic factors influencing the vector competence of ticks for *B. burgdorferi* have not been identified, growth-promoting factors may be present in *Ixodes* vectors that are absent in other tick genera, or
conversely, growth-inhibiting factors may occur in other tick genera that are lacking in *Ixodes* species.\(^9\)

As an initial step toward elucidating intrinsic factors that may be responsible for the vector competence of *I. pacificus*, we compared the dissemination of *B. burgdorferi* in this tick with that in *D. occidentalis* after introducing spirochetes via a capillary-feeding technique.\(^20\) We sought to determine the susceptibility and survivability of both species of ticks after ingestion of spirochetes to discover the route of spirochetal dissemination in infected ticks fed to partial repletion on rabbits versus unfed ticks. Also, we determined whether capillary-infected ticks are capable of transmitting spirochetes to rabbits.

**MATERIALS AND METHODS**

**Lyme spirochetes**

*B. burgdorferi* isolate CA4 (passage 6), which was derived from an adult *I. pacificus*, was used to infect laboratory-reared and spirochete-free ticks with the capillary feeding technique. The antigenic and genetic characteristics of this isolate have been described previously.\(^21,22\)

The capillary feeding method was modified slightly from that described by Burgdorfer.\(^20\) In brief, a nonheparinized capillary tube (75 mm long by 1.4 mm in diameter) was heated medially, and the two ends of the tube were drawn apart gently to create a narrow central portion. The medial section was then broken to fashion two capillary tubes having diameters of about 1.4 mm and 0.6 mm at their respective nonheated and heated ends.

The spirochetal suspension was prepared by inoculating known quantities of motile spirochetes into antibiotic-free BSK-II medium and adding adenine triphosphate to a concentration of 1.5 mM. A Petroff-Haussner counting chamber (Haussner Scientific Partnership, Horsham, Pa) was used to count spirochetes. After the capillary tube was embedded horizontally on a plastinect cuboid (about 10×10×4 mm), the hypostome of the tick was inserted into the narrow (0.6 mm diameter) end of the tube. The apparatus was put in a covered petri dish lined with moistened filter paper and held at 34°C for 2 to 3 hours while the ticks fed. Ticks gained up to 50% of their prefed weights during the feeding period. Ticks that gained less than 30% of their prefed weights were not used in the trials described subsequently.

**Source of ticks**

Host-seeking adults of *I. pacificus* and *D. occidentalis* were collected from low vegetation in Tilden Regional Park, Berkeley, Calif, with a 1-m\(^2\) tick-drag composed of white flannel. We used ticks from Tilden Regional Park because of their easy accessibility and the fact that the adults from there have rarely (*I. pacificus*) or never (*D. occidentalis*) been found to contain spirochetes.\(^11\)

Prior to experimentation, ticks were maintained at 21°C in a light:dark cycle of 12 hours:12 hours and relative humidities of 95% (*D. occidentalis*) or 98% (*I. pacificus*) for 1 to 2 weeks. Only motile ticks that seemed healthy were used in the capillary feeding trials.

**Effects of artificial feeding**

To compare the survivability of fed and unfed females, 48 *I. pacificus* were capillary fed BSK-II culture medium containing 2.8×10\(^8\) spirochetes per mL; 40 unfed females served as controls. Likewise, 113 *D. occidentalis* females were fed identically and 45 females were not fed as controls. In addition, 59 *D. occidentalis* females were fed only culture medium because a preliminary trial revealed that females fed a spirochetal suspension experienced a higher mortality than ticks that were not fed. All ticks were placed inside plastic vials (about 8 ticks/vial), which were held at the same conditions noted previously. The number of ticks that died monthly was recorded.

To determine the approximate number of spirochetes that were imbibed by each species of tick, 30 *I. pacificus* and 50 *D. occidentalis* females were weighed separately before and after capillary feeding. The average weight gains were 0.67 mg (SD=0.99) for *I. pacificus* and 1.004 mg (SD=3.78) for *D. occidentalis*. Because the density of BSK-II medium is 1.0176 g/mL, each *I. pacificus* and *D. occidentalis* female imbibed, on average, approximately 1.9×10\(^5\) and 2.7×10\(^5\) spirochetes, respectively.

The survival of capillary-fed versus unfed nymphal ticks also was determined. The survivability of 22 fed versus 17 nonfed *D. occidentalis* nymphs and of 62 fed and 16 nonfed *I. pacificus* nymphs was monitored weekly instead of monthly because capillary-fed nymphs had higher mortality rates than adult ticks. The amount of feeding material imbibed by a nymphal tick could not be determined because the weights of nymphs before and after feeding were too small to measure accurately. Spirochetes were delivered to *D. occidentalis* nymphs in 8% filtered rabbit serum instead of BSK-II medium because many *D. occidentalis* adults died after imbibing BSK-II medium alone. Nymphs were held at either 98% (*I. pacificus*) or 95% (*D. occidentalis*) relative humidity.

**Persistence of spirochetes in adult ticks**

To determine how long *B. burgdorferi* could survive in capillary-fed *I. pacificus* adults, the hemolymph and midgut diverticula of ticks were examined for the presence of spirochetes by direct immunofluorescence according to previously described methods at 12 days, 3 months, and 5 months after feeding.\(^23\) Also, portions of the midgut and salivary glands were put into BSK-II medium in isolation attempts. *D. occidentalis* adults were
likewise examined at 2, 4, and 5 months after feeding. To minimize contamination from other tissues, especially the midgut diverticula, the salivary glands were washed four times with phosphate-buffered saline before placement in BSK-II.

Transmission of spirochetes
Sixty days after capillary feeding, two spirochete-infected I. pacificus females and two male ticks that were not infected were put on each of four female New Zealand white rabbits inside feeding capsules. An identical trial was conducted with D. occidentalis. All eight rabbits were assayed for spirochetal infection by tick-xenodiagnosis 4 weeks after infected adult ticks had fed to repletion on them. About 30 spirochete-free I. pacificus larvae or 20 D. occidentalis larvae from laboratory colonies were put on the rabbits that had been exposed to infected adult I. pacificus or D. occidentalis. After repletion and drop-off, xenodiagnostic ticks were tested for spirochetes by direct immunofluorescence and by culturing their tissues 4 to 5 weeks after the transstadial molt. Moreover, six ear-punch biopsies (4 mm in diameter, three per ear) and three pieces of skin from adult tick feeding sites were put into BSK-II medium. Serum obtained from each rabbit 6 weeks after infected adult ticks fed on them was tested for antispirochetal antibodies by indirect immunofluorescence. The B-31 strain of B. burgdorferi was used as antigen.

The infectivity of capillary-fed nymphs of I. pacificus and D. occidentalis for rabbits also was tested. The density of spirochetes that nymphs were fed was $1.4 \times 10^{9}$/mL. Because capillary-fed nymphs of both species experienced a higher mortality rate than adult ticks, we placed fed nymphs on rabbits within 24 hours after they had ingested spirochetes. After repletion on rabbits and the subsequent transstadial molt, the midguts, hemolymph, and salivary glands of the resultant I. pacificus (n=15) and D. occidentalis (n=12) adults were examined for spirochetes by direct immunofluorescence and by culturing them in BSK-II medium. To determine if nymphal ticks transmitted spirochetes while feeding, ear-punch biopsies and serum obtained from each rabbit 5 weeks after tick detachment were assayed for infection, as described previously.

Spirochetal dissemination after a blood meal
To ascertain the course of spirochetal dissemination in adult I. pacificus after blood feeding, 20 infected female and 20 noninfected male ticks were put inside four feeding capsules (five females/five males per capsule) on each of four rabbits 3 to 4 weeks after capillary feeding. Five or 6 days after the ticks had attached, the partially fed ticks were removed manually from each rabbit.

Because D. occidentalis females experienced such a high mortality after capillary feeding, adults were put on rabbits 3 days after feeding rather than several weeks later as was done in the case of I. pacificus. Before placement on rabbits, adult ticks were exposed to one of three spirochetal densities ($4.6 \times 10^6$/mL, $1.7 \times 10^7$/mL, or $9.7 \times 10^7$/mL). Female ticks in these groups imbibed, on average, approximately $4.5 \times 10^5$, $1.7 \times 10^5$, and $9.6 \times 10^4$ spirochetes. Four feeding capsules were fastened on each of four rabbits to confine ticks. One rabbit received 28 ticks infected with about $4.5 \times 10^5$ spirochetes apiece, another rabbit received 24 ticks infected with about $1.7 \times 10^5$ spirochetes each, and the remaining two rabbits each received 12 ticks infected with about $9.6 \times 10^4$ spirochetes apiece. Partially replete ticks were removed manually on either the sixth or seventh day after attachment. Ticks whose hypostomes were damaged during removal were discarded. Partially fed I. pacificus or D. occidentalis females were dissected immediately after they were removed from animals. Tick tissues and hemolymph, and rabbit ear-biopsy tissues or sera, were assayed for spirochetal infection by the same methods outlined previously for the first series of experiments.

Data analysis
Fisher's exact test (two-tailed) was used to test for differences in prevalence of infection or mortality rates.

RESULTS
Survival of capillary-fed adult and nymphal ticks
The mortality rates of capillary-fed I. pacificus females at 30 and 60 days after feeding did not differ significantly from those of the nonfed controls ($P=0.13$).
and 0.91, Fig 1). *D. occidentalis* females fed BSK-II medium alone or BSK-II medium plus spirochetes experienced significantly higher mortality rates than nonfed ticks (*P*<0.001), but no difference in mortality rates was seen between females fed BSK-II only and those fed BSK-II plus spirochetes at 30 or 60 days after feeding (*P* = 0.35 and 0.87). Among nymphal ticks, significantly more capillary-fed *I. pacificus* and *D. occidentalis* died at 6 and 21 days after feeding than nonfed nymphs (*P*<0.01). Mortality between capillary-fed nymphs of *I. pacificus* and *D. occidentalis* did not differ significantly (Fig 2).

**Survival of spirochetes in capillary-fed adult ticks**

The midguts of all *I. pacificus* and *D. occidentalis* females were found to contain spirochetes 5 months after capillary feeding. Among *I. pacificus* females, the prevalence of hemolymph-test-positive ticks at 12 days (1/14, 7.1%) versus 3 months (1/15, 6.7%) after feeding did not differ significantly. The prevalence of infection in salivary glands washed in phosphate-buffered saline 12 days (1/14, 7.1%) and 3 months (2/17, 11.8%) after feeding was also comparable. In contrast, nonwashed salivary glands were more likely to be infected than those that were washed 3 months after capillary feeding (*P* = 0.0056, Table 1).

Except for one hemolymph-positive *D. occidentalis*, spirochetes were not detected in the hemolymph or salivary glands of capillary-fed *D. occidentalis* (Table 1).

**Transmission of spirochetes to rabbits by capillary-infected ticks**

Two of four rabbits exposed to spirochete-infected *I. pacificus* females seroconverted; one of these rabbits also tested positive by ear-punch biopsy, and the other yielded infected xenodiagnostic ticks (Table 2). Overall, 24% of the xenodiagnostic ticks that had fed as larvae on both infected rabbits acquired and transstadially passed spirochetes. In contrast, none of four rabbits fed upon by capillary-fed *D. occidentalis* females became infected (Table 2).

In trials involving capillary-infected nymphal ticks, 15 of 50 (30%) *I. pacificus* and 12 of 64 (19%) *D. occidentalis* put on separate rabbits fed to repletion and later molted. Three (20%) of the resultant *I. pacificus* and none of the *D. occidentalis* adults contained spirochetes in their midguts. Neither rabbit was found to be infected by ear-punch biopsy or indirect immunofluorescence examination of their sera (Table 3).

**Spirochetal dissemination during blood feeding**

The prevalence of spirochetal infection in capillary-fed female *I. pacificus* (n=15) and *D. occidentalis* (n=6) before blood feeding was 100%. Five or 6 days after attaching to rabbits, the midguts of 20 *I. pacificus* females were positive by direct immunofluorescence, but only 1 of 15 females tested concurrently by culture yielded spirochetes. The prevalence of spirochetal infection in *D. occidentalis* females that had been fed one of three dosages of spirochetes was significantly lower (*P*<0.001) than that of *I. pacificus* (Table 4), but the *D. occidentalis* females did not differ significantly among themselves (0.53<*P*<1). None of the midguts from *D. occidentalis* females produced positive cultures, regardless of the spirochetal dosage to which they had been subjected.

Hemolymph from 20 of 49 (41%) female *I. pacificus* were direct-immunofluorescence-test positive, and washed salivary glands from 3 of 10 (30%) ticks and 1 of 15 (7%) ticks were positive, respectively, by direct immunofluorescence and culture. Spirochetes were not detected in the hemolymph or salivary glands of *D. occidentalis* females. Cultures of ear-punch biopsies of rabbits fed upon by either species of tick did not yield spirochetes; however, all four rabbits fed upon by *I. pacificus* seroconverted.

**DISCUSSION**

The capillary-feeding method is a simple technique for experimentally infecting ixodid ticks with microbial agents for use in vector competence studies. Burgdorfer20 used a modified version of the glass capillary tube technique developed by Chabner26 to infect several species of ticks with microbial agents including the spirochete *Leptospira pomona*. Since then, European investigators have used this method to evaluate the vector efficiency of two *Ixodes* ticks for the Lyme disease spirochete.27-29 Here we report for the first time the use of the capillary-feeding method to evaluate the factors
Table 1
Prevalence of Spirochetal Infection in Various Tissues of D. occidentalis and I. pacificus Adults at Various Intervals After Capillary Feeding

<table>
<thead>
<tr>
<th>Species</th>
<th>Time after feeding (days)</th>
<th>No. positive (%) by direct immunofluorescence</th>
<th>No. positive (%) by culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Midgut</td>
<td>Hemolymph</td>
</tr>
<tr>
<td>I. pacificus</td>
<td>12</td>
<td>15/15</td>
<td>1/14</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>17/17</td>
<td>1/15</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>3/3</td>
<td>Not available</td>
</tr>
<tr>
<td>D. occidentalis</td>
<td>60</td>
<td>11/11</td>
<td>1/11</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>1/1</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>4/4</td>
<td>0/4</td>
</tr>
</tbody>
</table>

*Tissues were tested for spirochetes by direct immunofluorescence or by culture in BSK-II medium.

Table 2
Infectivities of Capillary-fed I. pacificus and D. occidentalis Females for New Zealand White Rabbits

<table>
<thead>
<tr>
<th>Tick species</th>
<th>Rabbit no.</th>
<th>Indirect immunofluorescence (Serum)</th>
<th>Culture (Ear-punch biopsy)</th>
<th>Tick xenodiagnosis†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive by</td>
<td></td>
<td>No. positive/no. tested (% positive)</td>
</tr>
<tr>
<td>I. pacificus</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>0/20 (31.6)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+</td>
<td>—</td>
<td>8/19 (42.1)</td>
</tr>
<tr>
<td>D. occidentalis</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>0/17 (5.3)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>0/10 (0)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>0/10 (0)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>—</td>
<td>—</td>
<td>0/16 (0)</td>
</tr>
</tbody>
</table>

†After the traumastidal molt, the midgut diverticula of xenodiagnostic nymphs were assayed for spirochetes using direct immunofluorescence or culture in BSK-II medium.

that may affect the efficiency of a proven competent (I. pacificus) versus that of an incompetent vector (D. occidentalis) for B. burgdorferi. The Western black-legged tick, I. pacificus, is a competent experimental vector of B. burgdorferi and the primary bridge vector to humans in the far Western United States. Unlike D. occidentalis, which is incapable of acquiring and transstadially passing Lyme disease spirochetes, I. pacificus can efficiently acquire, maintain, and transmit B. burgdorferi after having fed on naturally or experimentally infected rodents.

Our findings corroborate those of previous studies and suggest that the vector incompetence of D. occidentalis may be due to a midgut barrier to spirochetal dissemination, to physiologic changes during blood feeding that are inimical to spirochetes, or to both factors. Thus, spirochetes imbibed by I. pacificus females during capillary feeding disseminate to the salivary glands via the hemolymph in about 7% of ticks within 12 days. Further, if such ticks are placed on rabbits, 30% to 40% develop disseminated infections within 5 or 6 days of attachment. Similarly, Ixodes ricinus females infected via the capillary method develop systemic infections within a few days after their placement on rabbits. For instance, spirochetes were detected in the hemolymph of 2 of 11 I. ricinus capillary-infected females 2 days after attachment to rabbits and in the salivary glands of 6 of 51 ticks after 3 days of attachment.

In marked contrast, spirochetes in the midgut diverticula of artificially fed D. occidentalis females do not disperse to other tissues with or without a subsequent blood meal, which portends the existence of a midgut
Table 3
Spirochetal Prevalence in Various Tissues of I. pacificus and D. occidentalis Females Infected
By Capillary Feeding Before Partial Engraftment on New Zealand White Rabbits

<table>
<thead>
<tr>
<th>Tick species</th>
<th>No. (%) positive by direct immunofluorescence</th>
<th>No. (%) positive by culture in BSK-II</th>
<th>Infectivity for rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Washed</td>
<td>Washed</td>
<td>Midgut</td>
</tr>
<tr>
<td></td>
<td>salivary glands</td>
<td>Hemolymph</td>
<td>glands</td>
</tr>
<tr>
<td>I. pacificus</td>
<td>3/15 (20)</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>D. occidentalis</td>
<td>0/12</td>
<td>0/12</td>
<td>0/12</td>
</tr>
</tbody>
</table>

*Front legs were amputated to obtain hemolymph, and the amputated legs were cultured in BSK II medium in isolation attempts.

Table 4
Tissue Tropisms of B. burgdorferi in Capillary-fed I. pacificus and D. occidentalis Females Within 5 to 7 Days After Attachment to New Zealand White Rabbits

<table>
<thead>
<tr>
<th>Tick species (Spirochetal dose)*</th>
<th>No. (%) positive by direct immunofluorescence</th>
<th>No. (%) positive by culture in BSK-II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Washed</td>
<td>Washed</td>
</tr>
<tr>
<td></td>
<td>salivary glands</td>
<td>Hemolymph</td>
</tr>
<tr>
<td>I. pacificus (high)</td>
<td>20/20 (100)</td>
<td>20/49 (40.8)</td>
</tr>
<tr>
<td>D. occidentalis (high)</td>
<td>1/20 (5)</td>
<td>0/20</td>
</tr>
<tr>
<td>D. occidentalis (medium)</td>
<td>2/15 (13.3)</td>
<td>0/15</td>
</tr>
<tr>
<td>D. occidentalis (low)</td>
<td>2/12 (16.7)</td>
<td>0/12</td>
</tr>
</tbody>
</table>

*Ticks were fed one of three spirochetal densities: 4.6×10⁹/mL (high), 1.7×10⁹/mL (medium), or 9.7×10⁷/mL (low).
†Front legs were amputated to obtain hemolymph, and the amputated legs were cultured in BSK II medium in isolation attempts.

barrier. Also, the fact that most D. occidentalis females lose their midgut-restricted spirochetal infections by the fifth to seventh day after attachment indicates that intrinsic physiologic changes during the feeding process may be destructive to spirochetes.

We postulate therefore that D. occidentalis contains a borreliaclial factor that is released into the midgut diverticula during tick feeding, either from the salivary glands or from enzymes released by secretory cells lining the midgut. Other investigators have shown that many complex physiologic and biochemical changes occur in the midgut of ticks during blood feeding. For example, studies on the digestive enzymes of ticks demonstrated that protease is present in the digestive cells or lumen of the midgut. If some midgut enzymes induced during blood feeding are species-specific, either quantitatively or qualitatively, and are detrimental to spirochetes, they may contribute to the vector incompetence of certain ticks. Moreover, the viability of B. burgdorferi varies when incubated with cell cultures derived from different tick species.

Dermacentor variabilis and Amblyomma americanum can acquire B. burgdorferi while feeding on infected rodents, but both ticks are incapable of maintaining spirochetes or transmitting them to susceptible animals. The limited viability of spirochetes in these ticks after ingestion of blood is similar to that of D. occidentalis in the present study.

Although cultured spirochetes are widely used in experimental studies, the repertoire of surface proteins and the associated infectivity of cultured versus uncultured B. burgdorferi are not identical. To minimize this potential shortcoming, we used low-passaged spirochetes and treated both ticks similarly during experimentation. Even so, spirochetes in adult I. pacificus and D. occidentalis behaved differently while these ticks fed on rabbits, and only I. pacificus transmitted B. burgdorferi.

Nymphal ticks of both species suffered higher mortality rates than adult ticks when fed spirochetes artificially, whereas BSK-II medium alone adversely affected the survivability of adult D. occidentalis. Consequently, experiments with the nymphal ticks and adult D. occi-
dentalis need to be repeated using an innocuous diluent (e.g., defibrinated mouse blood) and lower doses of spiri-
chutes. We used inocula containing approximately $10^8$
ells/mL to infect ticks, which resulted in adult ticks
ingesting more than $10^8$ spinifera.

In comparable vector competence studies with European vector ticks, *I. ricinus* and *Ixodes hexagonus*,
Gern and coworkers exposed ticks to lower concentra-
tions of spinifera ($10^7$ to $10^8$ cells/mL) cultured in
BSK-II medium with no apparent untoward effects.

In comparison, naturally or experimentally infected
vectors of *B. burgdorferi* contain only about $10^3$
spirochete in the uninfected state. For example, the mean
number of spinifera present in experimentally infected
*I. dammini* (now *I. scapularis*) was estimated to be less
than 300 for nymphal and about 4000 for adult ticks,
and the median density of *B. burgdorferi* in naturally
infected adult ticks from intensely zoonotic sites in
coastal Massachusetts was 192.5. Further experimental
studies are necessary to determine the minimum thresh-
old of spinifera that various enzootic or bridge vectors
must ingest to acquire, transstadially pass, and transmit
*B. burgdorferi*.

We conclude that the capillary-feeding method is
highly suitable for this purpose because previously cul-
tured and characterized microbial agents can be effi-
ciently delivered to ticks in known concentrations with
minimal expense and effort.

Acknowledgments: We thank Esther Omí-Olsen for
her technical assistance.

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The Role of Publicly Owned Properties in the Transmission of Lyme Disease in Central New Jersey

Terry L. Schulze, PhD and Robert A. Jordan, PhD

ABSTRACT

Using an ecological index to generate a relative ranking of sites regarding potential and actual Lyme disease transmission risk, 610 public parks, recreation areas, and public school properties were surveyed during the summer of 1993. The majority of surveyed sites (56.4%) were judged to pose low potential risk; only 60 sites (9.8%) were identified as posing a high potential risk, requiring additional assessment to estimate actual population densities of infected ticks.

Key words: public parks, recreation areas, Lyme disease

INTRODUCTION

Despite general acceptance that the majority of Lyme disease cases are the result of exposure to infected black-legged ticks at or near the patient's place of residence, other studies have suggested substantial transmission risk among visitors, workers, and nearby residents of some parks and recreation areas. None of these studies adequately characterized habitats and use within the areas studied and, owing to the labor intensity inherent in surveying large areas for ticks, the number of study sites was limited. As a result, the majority of parks and recreational areas are never assessed, leaving the public unaware of potential risk. No studies of transmission risk associated with school properties have been published.

Recognizing the limitations resulting from the level of effort required to perform tick surveys, an ecological assessment index (Index) was designed to predict potential Lyme disease transmission risk based on the presence, amount, and accessibility of vegetation associations capable of supporting Ixodes scapularis Say and its hosts. The results of that pilot study suggested that the Index provides a rapid, accurate method to identify areas at risk for the transmission of Lyme disease.

Through a broad-scale assessment program, the study attempted to test and refine the efficacy of the Index throughout a county where Lyme disease is endemic. Such research is the first step toward the creation of cost-effective management procedures to reduce the incidence of Lyme disease in people using public properties by reducing exposure to infected I. scapularis. We report here the results of a survey of all public land in Monmouth County, NJ, for Lyme disease transmission risk.

METHODS

Site selection

Publicly owned land in Monmouth County, NJ, served as study areas for this project. Since Lyme disease became reportable in New Jersey in 1980,
Monmouth County has accounted for approximately 22% of all reported cases statewide through 1989. The overall goal of the project was to assess the risk of transmission on all publicly owned lands in Monmouth County as a first step in a county-wide program of Lyme disease prevention and control. Included in the survey were all municipal- and county-administered parks and recreation areas, local- and county-owned open space, state-administered wildlife management areas (WMA) and parks, and federally owned lands. In addition, athletic fields and other open spaces associated with all public schools were included in the surveys.

Habitat of *Ixodes scapularis* in Monmouth County, NJ

*I. scapularis* tends to be most prevalent in wooded areas and associated ecotones and in old fields in secondary woody succession. In the coastal plain of New Jersey, where the majority of Lyme disease cases have been reported, habitats that support high densities of ticks are comprised of mixed hardwood (*Quercus alba, Quercus prinus, and Quercus rubra*); mixed hardwood/pine (*Pinus rigida*); and pine forests with a dense shrub layer dominated by highbush blueberry (*Vaccinium corymbosum*), lowbush blueberry (*Vaccinium angustifolium*), and huckleberries (*Gaylussacia* spp.). Minor species generally include sheep laurel (*Kalmia angustifolia*), northern bayberry (*Myrica pensylvanica*), and often dense tangles of common greenbriar (*Smilax rotundifolia*).

Brush/scrub habitats (late old fields) also may serve as adequate habitat, as do certain ornamental landscappings. Suburban residential foci for Lyme disease in the Northeast frequently are associated with adjacent or nearby woodlands. The presence of dense shrub layers, leaf litter, and other plant debris seems to play an important role in the survival of subadult ticks by maintaining conditions of high humidity. Thus, habitats generally unfavorable for ticks include open sunny areas such as turfgrass (lawns, athletic fields, and other recreational areas), agricultural land, and wetlands.

Ecological assessment Index

The assessment Index used in this study relies on documented habitat affinities of *I. scapularis*, the black-legged tick (formerly *I. dammini*), the primary vector for the Lyme disease spirochete *Borrelia burgdorferi* in the Northeast. Assessment of potential risk relies on evaluation of plant communities associated with a particular site. Because potential risk also is dependent on human exposure, the Index also considers the degree to which suitable tick habitat is accessible to people potentially at risk. The assessment of actual risk of transmission incorporates *I. scapularis* abundance and rate of infection by *B. burgdorferi*.

Assessment of potential risk

Potential risk was evaluated based on the characterization of sites with respect to three parameters that describe the suitability of vegetation as tick habitat, the extent of that habitat, and its accessibility to people using the site:

1. The habitat suitability parameter addresses the relative suitability of available plant cover types for the support of *I. scapularis* populations. A numeric value is assigned, ranging from 1 (agricultural fields and lawns) to 5 (mature forest with substantial shrub layer).

2. Amount of tick habitat on a given property is represented as a percentage of the total area of the property. Point values for habitat availability range from 1 (<20% of the property is tick habitat) to 5 (80% to 100% of the property is tick habitat).

3. Accessibility of tick habitat recognizes that although tick habitat may be present on a particular site, its presence poses no risk if people do not or cannot access it and thus become exposed to infected ticks. Relative accessibility is ranked on a scale from 1 (no access or no suitable tick habitat present) to 5 (suitable tick habitat easily accessible or access is encouraged).

Availability of habitat for ticks may be assessed throughout the year, whereas the seasonality of tick activity restricts use of the simple presence or absence of a given stage of *I. scapularis* to a particular time of year. Owing to the relative ease in collection and high infection rates in adults compared with immature *I. scapularis*, assessment of tick abundance is logistically limited to the early spring and fall in New Jersey. Thus, the Index permits characterization of potential risk apart from the presence of ticks and can be performed at any time to determine whether additional action is necessary.

Point values, ranging from a minimum of 1 to a maximum of 5, are assigned to each of the three parameters and totaled. The resulting score is used to determine the appropriate response action for each site and the need to proceed to the second phase of the survey to assess actual risk (Table 1). Sites ranked as having either a moderate or high potential risk of Lyme disease infection are surveyed for actual risk according to a predetermined numeric priority.

Assessment of actual risk

Actual risk is evaluated using data on the presence and size of the tick population and its rate of infection with *B. burgdorferi*. Sites were surveyed for questing adult *I. scapularis* during periods of peak adult activity in October and November 1993. Adult ticks were chosen for use in the Index because of their demonstrated high rate of infec-
Suitable habitats at all sites were evaluated systematically using walking surveys. All surveys were performed by the same personnel (to avoid sampling biases) on clear days between 10 AM and 2 PM when peak activity was expected. Areas were surveyed for a 60-minute period. At smaller sites, the survey time was reduced as appropriate and the number of ticks collected was extrapolated to the 60-minute standard for ease of comparison.

This survey technique provides a fairly accurate representation of the expected number of adult ticks encountered by humans. The methodology, however, may be modified to permit collection of subadults. The relative abundance of questing ticks was ranked using numeric values ranging from 1 (0 ticks/survey) to 5 (>30 ticks/survey).

Ticks collected during surveys were retained and evaluated for the presence of *B. burgdorferi*. Ticks were dissected and midguts were triturated in phosphate-buffered saline solution on microscope slides. A maximum of 50 fields were examined at ×400 magnification to calculate minimum field infection rates. Field infection rates of collected ticks were ranked using numeric values ranging from 1 (0% to 9% of ticks infected) to 5 (>40% of ticks infected). Point values for potential risk (tick habitat suitability, amount, and accessibility) are added to the point values for tick abundance and infection rate to obtain the actual risk scores used to establish priorities for surveillance and intervention strategies (Table 2).

**Table 1**

<table>
<thead>
<tr>
<th>Scoring range</th>
<th>Description of risk</th>
<th>Response action</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 to 15</td>
<td>High</td>
<td>Place under routine tick surveillance and establish rates of infection.</td>
</tr>
<tr>
<td>6 to 10</td>
<td>Moderate</td>
<td>Consider periodic tick surveillance and establishment of infection rates.</td>
</tr>
<tr>
<td>&lt;6</td>
<td>Low</td>
<td>No action necessary at this time or any time in the future.</td>
</tr>
</tbody>
</table>

**Table 2**

<table>
<thead>
<tr>
<th>Scoring range</th>
<th>Description of risk</th>
<th>Response action</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 to 25</td>
<td>Definite risk</td>
<td>Take immediate action (post area, devise control strategy).</td>
</tr>
<tr>
<td>16 to 20</td>
<td>Potential risk</td>
<td>Consider action as above, place under routine tick surveillance.</td>
</tr>
<tr>
<td>11 to 15</td>
<td>Limited risk</td>
<td>Perform periodic tick surveillance.</td>
</tr>
<tr>
<td>6 to 10</td>
<td>No present risk</td>
<td>Consider periodic tick surveillance; no action necessary.</td>
</tr>
<tr>
<td>&lt;6</td>
<td>No risk likely</td>
<td>No action necessary at this time or at any time in the future.</td>
</tr>
</tbody>
</table>

Potential risk of transmission

Within Monmouth County’s 52 municipalities, 610 sites were surveyed for potential risk of transmission, including 415 municipal parks and recreation areas, 27 county parks, seven state parks and WMAs, three federal properties, and 158 school properties (Table 3). Sites ranged in size from less than 1 acre to the 16-mile² Naval Weapons Station Earle (NWS Earle). Excluding NWS Earle and Fort Monmouth, a total of 26,923 acres of publicly held parks and recreation areas was surveyed. Acreage of school properties was not available.

A total of 58 (12.8%) parks and recreation areas demonstrated high potential risk of transmission; 136 (30.1%) parks were assigned moderate risk; and the remaining 258 (57.1%) parks and recreational areas received a low rating for potential risk. Of the public school properties, only two (1.3%) demonstrated high potential risk of transmission; 70 (44.3%) were assigned moderate risk; and the remaining 86 (54.4%) received a low risk rating.

**Actual risk of transmission**

Under normal circumstances, the Index requires survey of both high and moderate potential risk sites. Owing to the magnitude of the study and resource constraints, however, only high potential risk sites were surveyed to determine actual risk. A total of 60 sites (9.8% of all sites) in Monmouth County received numeric scores that suggested high potential risk for Lyme disease transmission. Of the 60 sites in the high potential risk category, 34 were either undeveloped or no longer being used, and therefore were not considered for additional assessment. The remaining 26 sites subsequently were surveyed to assess the level of actual risk to design future surveillance activities and intervention strategies.

Ticks were collected from 11 of 24 parks and recreation areas surveyed for actual risk of transmission. In 60-minute surveys, the sites yielded between 3 and 53 ticks (mean=20.2 ticks/60-minute survey). Minimum field infection rates ranged between 0 and 50% (mean=32.9%). As a result of tick abundance and infection rate data, three of the parks and recreation areas (12.5%) were classified as definite risk, 11 (45.8%) as potential risk, and 10 (41.7%) as limited risk. Tick collections were made at only two of the 158 schools (1.3%) surveyed. No ticks were collected from either site during 60-minute surveys and both were classified as posing
DISCUSSION

The majority of sites (344, or 56.4% of all sites) ranked low in potential transmission risk and required no further survey efforts. Although human resource constraints prevented further assessment of moderate potential risk sites, these public properties likely will require additional attention as the goals of local authorities and responsible agencies warrant. Surveillance of tick populations is a labor-intensive and time-consuming enterprise. Therefore, depending on available human resources and local understanding of the relative degree that different public areas tend to be used, assessment personnel must prioritize sites using relative potential risk. For example, sites that score a potential risk value of 9 or 10 should be addressed with greater urgency than sites scoring 6 or 7 when allocating resources for subsequent assessments for actual risk of transmission. Local officials can best assess whether habitat suitability, accessibility of existing tick habitat, or another of the measured parameters actually contributes most to transmission risk at a particular site. Local knowledge of public land can be quite useful in developing intervention or management strategies.

Some areas yielded no ticks during surveys, yet were classified as demonstrating high potential risk. This apparently anomalous result is explained by the fact that the Index relies both on the actual presence and infection rates of ticks and on the suitability of available habitats to support tick populations. For example, a particular park that scored high with regard to potential risk may be assigned limited actual risk if surveys fail to produce many infected ticks. The high score resulting from the assessment of the habitat parameters, however, suggests that the potential for ticks to be present in subsequent years is significant and underscores the need for periodic surveillance. Even relatively low tick density can generate high transmission risk.

In other instances, sites yielding higher numbers of ticks were ranked lower in actual risk of transmission than sites with fewer ticks. For example, NWS Earle yielded 46 ticks/60-minute survey and was classified as having potential risk, although Turkey Swamp Park yielded only 21 ticks/60-minute survey and was classified as a definite risk site. Turkey Swamp Park ranked higher in potential risk of transmission because of a high accessibility score compared with NWS Earle, which is a secured facility with severe access restrictions.

During the course of this project, it became apparent that although the Index provided a flexible survey instrument allowing the rapid assessment of potential and actual risk of Lyme disease transmission over large geographic areas, some operational modification of the methodology would be required. The utility of the Index generally was limited to large tracts; primarily because as tract size increases, habitat diversity increases, and larger tracts tend to feature multiple-use facilities. The risk of exposure at larger parks, then, generally is use dependent. For example, park visitors accessing hiking trails that run through forested areas suitable as tick habitat will experience significantly greater risk of exposure to *I. scapularis* than those who limit their activity to athletic fields and paved courts.

Similarly, certain areas tend to be of limited use or used by a limited public constituency. For example, state WMAs are relatively undeveloped and generally are used by hunters and anglers rather than the public at large. Exposure to *I. scapularis* is, therefore, user group related. Consequently, any future assessment of risk transmission performed as part of an integrated management program should address the specific constituency at risk. For larger sites with more than one type of suitable tick habitat or use, and where some type of intervention is anticipated, the assessment of potential risk should address each habitat and use separately.

Data on the geographic distribution of public properties at risk for Lyme disease transmission suggest certain trends. In general, high-risk sites were limited in number and confined to the western portion of the county. Coastal towns consistently had the greatest number of low-risk municipal parks, recreation areas, and schools. Of the
Table 4
Summary of *Ixodes scapularis* Abundance and Infection Rates (IR) From Recreational Areas and School Properties Yielding Ticks in Monmouth County, NJ, and Resultant Actual Risk of Lyme Disease Transmission

<table>
<thead>
<tr>
<th>Municipality</th>
<th>Location</th>
<th>Number of ticks*</th>
<th>IR (%)</th>
<th>Relative risk†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colts Neck Township</td>
<td>NWS Earle</td>
<td>46</td>
<td>38.9</td>
<td>Potential risk</td>
</tr>
<tr>
<td>Eatontown Township</td>
<td>Wall Street Park</td>
<td>4</td>
<td>0</td>
<td>Potential risk</td>
</tr>
<tr>
<td></td>
<td>Woodmere School</td>
<td>0</td>
<td>-</td>
<td>Limited risk</td>
</tr>
<tr>
<td>Freehold Borough</td>
<td>Lake Topanemus</td>
<td>9</td>
<td>33.3</td>
<td>Potential risk</td>
</tr>
<tr>
<td>Freehold Township</td>
<td>Turkey Swamp Park</td>
<td>21</td>
<td>41.7</td>
<td>Define risk</td>
</tr>
<tr>
<td></td>
<td>Whittier Oaks Park</td>
<td>24</td>
<td>33.3</td>
<td>Define risk</td>
</tr>
<tr>
<td></td>
<td>Woodgate Farms</td>
<td>0</td>
<td>-</td>
<td>Potential risk</td>
</tr>
<tr>
<td></td>
<td>Freehold High School</td>
<td>0</td>
<td>-</td>
<td>Limited risk</td>
</tr>
<tr>
<td>Holmdel Township</td>
<td>Holmdel County Park</td>
<td>3</td>
<td>0</td>
<td>Limited risk</td>
</tr>
<tr>
<td>Howell Township</td>
<td>Allaire State Park</td>
<td>53</td>
<td>37.8</td>
<td>Define risk</td>
</tr>
<tr>
<td></td>
<td>Oak Glen Park</td>
<td>4</td>
<td>0</td>
<td>Limited risk</td>
</tr>
<tr>
<td>Manalapan</td>
<td>Deerway Mobile Park</td>
<td>0</td>
<td>-</td>
<td>Potential risk</td>
</tr>
<tr>
<td></td>
<td>Gordons Corner Park</td>
<td>0</td>
<td>-</td>
<td>Limited risk</td>
</tr>
<tr>
<td></td>
<td>Pinewood Drive Park</td>
<td>0</td>
<td>-</td>
<td>Potential risk</td>
</tr>
<tr>
<td>Middletown</td>
<td>Hartshorne County Park</td>
<td>0</td>
<td>-</td>
<td>Potential risk</td>
</tr>
<tr>
<td></td>
<td>Huber Woods County Park</td>
<td>0</td>
<td>-</td>
<td>Limited risk</td>
</tr>
<tr>
<td></td>
<td>Poricy Park</td>
<td>4</td>
<td>50.0</td>
<td>Potential risk</td>
</tr>
<tr>
<td></td>
<td>Tatum Park</td>
<td>0</td>
<td>-</td>
<td>Limited risk</td>
</tr>
<tr>
<td></td>
<td>Thompson Park</td>
<td>0</td>
<td>-</td>
<td>Limited risk</td>
</tr>
<tr>
<td>Millstone Township</td>
<td>Assunpink WMA</td>
<td>33</td>
<td>30.0</td>
<td>Potential risk</td>
</tr>
<tr>
<td>Neptune</td>
<td>Shark River County Park</td>
<td>21</td>
<td>14.3</td>
<td>Potential risk</td>
</tr>
<tr>
<td>Sea Girt</td>
<td>Crescent Park</td>
<td>0</td>
<td>-</td>
<td>Limited risk</td>
</tr>
<tr>
<td>Tinton Falls</td>
<td>Pinebrook Recreation</td>
<td>0</td>
<td>-</td>
<td>Limited risk</td>
</tr>
<tr>
<td>Wall Township</td>
<td>Marigold Park</td>
<td>0</td>
<td>-</td>
<td>Limited risk</td>
</tr>
<tr>
<td>West Long Branch</td>
<td>Wall Street Park</td>
<td>0</td>
<td>-</td>
<td>Limited risk</td>
</tr>
</tbody>
</table>

*Adult ticks/60-min survey.*
†Response actions recommended:

- **Definite risk**—take immediate action (post area, devise control strategy);
- **Potential risk**—consider action as stated above but with lower priority, place under routine tick surveillance;
- **Limited risk**—perform periodic tick surveillance.

One third (n=17) of municipal parks and recreation areas with the lowest mean scores, 15 (88.2%) were in coastal towns. Parks and recreation areas in these communities—primarily urban areas with high development intensity—tend to be of low suitability for the support of tick populations. Developed parks tend to be maintained lawns or beach areas; areas supporting woody vegetation tend to be maritime forest with poorly developed understory structure, which offers poor habitat for ticks. Demographic data show that the majority of the population in Monmouth County is concentrated in the shore communities; consequently, limited resources available for prevention and control activities can be more appropriately targeted to specific areas that demonstrate the highest risk of transmission.

Although most (54.4%) school properties demonstrated low risk, a significant number (n=70) were assigned moderate potential risk. A total of 61 of the 70 schools, however, received a score of "6," indicating that suitable tick habitat was located on private property adjacent to the school grounds. As such, prevention and control options may be limited.

Park managers had mixed reactions to the results of this study. Some felt that as only a small number of public properties posed any real risk of Lyme disease transmission, public concerns regarding the relative safety of parks, recreation areas, and schools should be allayed. Some managers of parks identified as having high risk, however, voiced concerns that a decrease in park use and loss of revenue would follow public disclosure of the study. Clearly, public health officials and park administrators should work in concert to develop ways to inform park visitors about habitats and behaviors that may reduce risk of exposure to infected ticks. Ideally, information obtained from these educational efforts will carry forward and have an impact on reducing peridomestic exposure.
Where intervention is deemed a next step at high-risk sites—which generally are large, multiple-use parks and recreation areas—tick management through the use of acaricides may be impractical and may not receive public support. Rather, the goal of prevention and control activities should be “exposure management,” where an integrated approach (education, posting, vegetation control, etc) is used to reduce transmission risk. Targeting areas most at risk for transmission of Lyme disease also will assist in directing educational efforts at human populations at risk. Awareness signs and other efforts may be most productively used in parks and recreational areas where the risk is highest and the user groups are more clearly defined.

Where the use of acaricides is deemed appropriate, control efforts directed against vector ticks should be limited to areas that provide suitable habitat for ticks, thus eliminating unnecessary applications and reducing the amount of acaricide placed into the environment. This is particularly important around schoolyards and other areas where children spend significant periods of time. Because acaricide use is minimized, the costs of intervention are reduced significantly.

Results of this project may be used to identify managed areas at risk for Lyme disease prospectively. Previously, areas at risk could be identified only retrospectively, either by randomly conducted tick surveys or by plotting locations of case reports. Use of the Index allowed the majority of public areas to be systematically eliminated from public health concern after initial assessment efforts indicated low potential risk for Lyme disease transmission because existing vegetative cover did not provide adequate habitat for I. scapularis.

REFERENCES


An author of “The Jarisch-Herxheimer Reaction in Patients with Erythema Migrans,” which appeared in the June 1996 issue, would like to add the following note to go along with Table 1: Single EM lesions were noted in 43 of 50 patients (86%) with Jarisch-Herxheimer reactions (JHR) and in 243 of 305 patients (80%) without JHR.
IgG Antibodies to Borrelia burgdorferi in Rodents in Tennessee

Thomas M. Kollars, Jr, PhD; Donald D. Ourth, PhD; Timothy D. Lockey, PhD; and Daniel Markowski, MS

ABSTRACT

Goat-anti white-footed mouse (Peromyscus leucopus) IgG was used in an enzyme-linked immunosorbent assay (ELISA) and a Western blot assay to test for borrelial antibodies in sera from cricetid rodents captured in Tennessee. The conjugate was cross-reactive between cricetids (New World rodents) but was weakly or not cross-reactive with murids (Old World rodents) and sciurids (squirrels). Using the ELISA, 9% of cotton rats (Sigmodon hispidus), 19% of woodland voles (Microtus pinetorum), 31% of white-footed mice (Peromyscus leucopus), 57% of golden mice (Ochrotomys nuttali), and 100% (one captured) of cotton mice (P. gossypinus) were positive for borrelial IgG antibodies; neither rice rats (Oryzomys palustris) nor Eastern harvest mice (Reithrodontomys humulis) were positive for borrelial IgG antibodies. Using Western blot, neither cotton rats or cotton mice were positive; 10% of woodland voles, 7% of golden mice, and 6% of white-footed mice were positive using the presence of 31 or 34 kDa bands present with other bands (15, 21, 26, 39, 41, 66, and 83 kDa). In rodents, unlike borrelial antibodies in raccoons from Shelby county, a high ELISA titer was not associated with a positive Western blot. In addition, the distribution of rodents with borrelial antibodies was independent of raccoons with borrelial antibodies, indicating that interaction between these mammal groups may not be a factor in animals becoming infected. The immunologic evidence reported in this study and the recent isolations of Borrelia burgdorferi in the southern United States indicates that further research on Lyme borreliosis in Tennessee is necessary, including isolation of the spirochete.

Key words: Borrelia burgdorferi, Lyme disease, antibodies, enzyme-linked immunosorbent assay, Western blot, Cricetidae, rodent

INTRODUCTION

Borrelia burgdorferi, the causative agent of Lyme disease, has been isolated from many wild mammals. B. burgdorferi was first isolated from white-footed mice and raccoons. Since then, a number of different wild mammals, birds, and ticks have been tested for presence of the spirochete or antibodies to B. burgdorferi using enzyme-linked immunosorbent assays (ELISA) and immunofluorescent assays (IFA). Magnarelli, Oliver, Hutcheson, and Anderson found ELISA to be more sensitive than IFA, and more suitable for testing numerous serum samples for antibodies to B. burgdorferi. In the southern United States, ELISA have been used to detect borrelial antibodies in sera.
from white-tailed deer (*Odocoileus virginianus*), cotton mice (*Peromyscus gossypinus*), white-footed mice (*Peromyscus leucopus*), and raccoons (*Procyon lotor*). Western blot analysis has been used to detect borrelial antibodies and confirm ELISA tests in humans and other mammals. No reports of borrelial antibodies in rodents have been made in Tennessee.

The Memphis and Shelby County Health Department began a tick research project in 1990 to ascertain the risk of Lyme disease to the human population in western Tennessee. One aspect of this project was to determine if rodents have antibodies to *B. burgdorferi*. Sera from rodents were tested for antibodies to *B. burgdorferi* using the ELISA and Western blot methods to determine if the potential for Lyme disease is present in Tennessee.

**METHODS**

**Study sites and sampling**

A total of 49 one-acre sites were selected for trapping rodents from June 1990 through August 1991. Within each site, 40 live traps (Sherman Trap Company) were placed in a line transect or grid, depending on topography. Captured rodents were euthanized with rompul, examined for ticks, and blood was collected by heart puncture. Serum samples were stored at -70°C until they were tested.

**Serologic tests**

The ELISA method was used with modifications. Microwell plates adsorbed with whole cell sonicated (WCS) *B. burgdorferi* strain B-31 were provided by Zeus Laboratories, Raritan, NJ. Goat-anti white-footed mouse (*P. leucopus*) IgG-peroxidase conjugate (Kirkegaard and Perry Laboratories, Gaithersburg, Md) was absorbed with diluent containing *Treponema phagedenis* (MHA-TP, Miles Laboratories, Elkhart, Ind) to reduce nonspecific binding (1:100 dilution). Goat-anti white-footed mouse IgG conjugate detected IgG of cricetid rodents but was not effective in detecting IgG from murid or sciurid rodents. Other researchers should note that the goat-anti white-footed mouse conjugate cross-reacted with the WCS, but with absorption with MHA-TP, all cross-reactivity ceased.

Dr. Louis Magnarelli (Connecticut Agricultural Research Station) graciously provided sera from two positive and one negative white-footed mouse control. Sera were screened at a dilution (in PBS-Tween) of 1:160, also used by other researchers. If positive, sera were serially diluted to an end titer or estimated using a standard curve based on positive controls. The substrate used was o-phenylenediamine (1,2-benzenediamine). Nine sera from white-footed mice testing negative by Western blot were used in addition to the negative control provided by Dr. Magnarelli. A net optical density (OD) ≥0.05 was considered positive for the diluted sera from rodents based on the mean OD of negative controls (0.02) plus three standard deviations (0.03). All sera testing positive by ELISA were then tested by Western blot analysis.

Western blot analysis was conducted to confirm all serum samples that tested positive using ELISA. Western blot analysis was conducted following Kollars, Ourth, and Lockey, using test strips from the Lyme Disease MAR- BLOT strip test system using *B. burgdorferi* strain B-31 (March Diagnostics Inc, Carlsbad, Calif). A serum sample was considered positive using the Mardx criteria from 1993 (31 and 34 kDa bands occurring together or one of these two bands occurring with at least one of the following bands: 25, 39, 41, or 83). Sera from the same positive and negative controls used in the ELISA tests were used as controls for Western blots. Statistical comparisons between the percent of rodents positive by ELISA and Western blot were conducted using Chi-square analysis and stepwise Bonferroni adjustment. Chi-square and Fisher’s exact test for small sample size were used to test whether the distribution of rodents positive by both ELISA and Western blot were independent of the distribution of raccoons found positive by both tests.

**RESULTS**

Sera from 37 of 170 individuals from seven cricetid rodent species (22%) tested positive for borrelial antibodies using ELISA. Titers ranged from 1:160 to 1:2560 for borrelialian antibodies. Using the ELISA, none of 17 rice rats (*Oryzomys palustris*), neither of two eastern harvest mice (*Reithrodontomys humilis*), 9% (4/47) of cotton rats (*Sigmodon hispidus*), 19% (4/21) of woodland voles (*Microtus pinetorum*), 31% (21/68) of white-footed mice (*P. leucopus*), 57% (8/14) of golden mice (*Ochrotomys nutalli*), and 100% (1/1) of cotton mice (*P. gossypinus*) were positive for borrelial IgG antibodies. No cotton rats or cotton mice were positive; 10% of woodland voles, 7% of golden mice, and 6% of white-footed mice were positive using the presence of 31 or 34 kDa band in individuals positive by ELISA tested by Western blot analysis. Seven rodents tested positive for borrelialian antibodies by Western blot analysis, representing 4% of total rodents tested (137) or 19% of positive ELISAs (37) (Table). The 41 kDa band was the most commonly found band using positive ELISA but negative Western blot serum data. No significant differences between the percentage positive by both tests with titers of 160 through 2560 (P>0.05) were found. Three of five areas of positive Western blot rodents co-occurred with three of six positive Western blot sites of raccoons. The distribution of rodents positive by both tests was independent of raccoons positive by both tests (χ²=0.11, Fisher’s P>0.05). A map of Shelby County showing sites from
which rodents tested positive using Western blot assay are shown in the Figure.

**DISCUSSION**

The ELISA results in this study (22% positive) were similar to results in other Eastern states. White-footed mice and cotton mice from other states in the southern United States were positive at 36% and 27%, respectively.\(^7\) Sera positive by the ELISA were confirmed using commercially available Western blot strips. Cross-reactivity of antibodies can occur in human disease; this also may be true for wild mammals.\(^14\) Serologic testing for *B. burgdorferi* may give false positive results due to shared antigens with other spirochetes.\(^17-19\) Although rare, cross-reactivity to 31 or 34 kDa bands (OspA and OspB, respectively) by serum antibodies to other diseases can occur in humans. Reactivity to both 31 and 34 kDa bands, however, only occurred in positive control patients.\(^20\) According to Hilton, Devoti, and Sood,\(^21\) the presence of 5 of the following 12 bands is considered a positive Western blot: 18, 21, 28, 30, 31, 34, 39, 41, 45, 58, 66, and 93 kDa. The 18, 21, 31, 34, 41, and 66 kDa bands were present in some rodents (Table); however, because of the controversial nature of Lyme disease in the southeastern United States and the lack of an isolate of *B. burgdorferi* in Tennessee, we chose a conservative approach.

The percentage of rodents having borreliarial antibodies was reduced (ELISA vs Western blot results) from 22% to 4% (Table). Few reports of Western blots of wild captured mammals have been reported. In deer, the presence of antibodies to 31 and 34 kDa proteins in Minnesota was 2% in experimentally inoculated deer.\(^13\) In Tennessee, 47% of raccoons tested positive by ELISA and 12% by Western blot using the 31 and 34 kDa criteria.\(^9\)

Although conservative criteria may exclude detecting antibodies to non-*Borrelia* species, antibodies against another or multiple *Borrelia* species may have been detected as indicated by the presence of other *B. burgdorferi* diagnostic bands.\(^21\) Apparent geographic variation and heterogeneity of *Borrelia* species exist in the United States. A *Borrelia* species was isolated from dogs in Florida\(^22\) and a new species (*B. andersonii*) has been described from rabbits collected in the eastern United States.\(^23\) In addition, phenotypic variation has been shown to occur in *Borrelia* isolates from Illinois\(^24\) and Missouri (personal observation, T.M.K.); mixed infection of different *Borrelia* species has been shown to occur in wild mammals\(^25\) and ticks (personal observation, T.M.K.). Isolates of *B. burgdorferi* from cotton mice and cotton rats have been made in the southern United States\(^26\): 1 of 45 *I. scapularis* was PCR positive using flagellin primer (personal observation, T.M.K.),\(^27\) and *B. burgdorferi* may be endemic in some southeastern states, unlike previous assumptions.\(^28\)

The use of local strains of *B. burgdorferi* does not appear to be required for optimal sensitivity of ELISA\(^29\).
however, differences in Western blots can occur when different strains are used for human sera.30 Different strains of *B. burgdorferi* also show the same ELISA (no variance) and Western blot (with variance) pattern in laboratory-inoculated white-footed mice.31

Positive titers were found in rodents from rural areas of Shelby County and urban areas within the city of Memphis and indicate that rodents are infected with *B. burgdorferi* or possibly some other *Borrelia* species. Similar reductions in the number of animals positive by ELISA and positive Western blots occurred in rodents and raccoons in Shelby County. The data indicate that in rodents, unlike borreliial antibodies in raccoons from Shelby County, a higher ELISA titer was not associated with a positive Western blot. In addition, the distribution of raccoons with borreliial antibodies is independent of rodents with borreliial antibodies. This indicates that interaction between raccoons and cricetid rodents is not necessary for animals to become infected; ie, ticks from mice are not necessarily needed to infect raccoons.

Human cases of Lyme borreliosis have been reported in Tennessee.32 Serologic testing of wild mammals such as raccoons and rodents can provide important information in surveillance programs37 and may indicate areas of increased risk for Lyme disease in Tennessee. We suggest that *B. burgdorferi* or another *Borrelia* spp. infects raccoons and rodents in western Tennessee. The occurrence of *B. burgdorferi* in the southeastern United States has been documented and isolates have been made from southeastern Missouri about 150 miles north of Memphis.33 Although tick species shown to be vectors of *B. burgdorferi* in other states34-38 have been collected in Shelby County,39-41 the isolation of *B. burgdorferi* from ticks or wild mammals is necessary to confirm the presence of this spirochete in Tennessee and is the focus of ongoing studies.

*Ixodes dentatus* commonly is found on rabbits in Shelby County and also has been found on a white-footed mouse in the county.43 This tick species probably plays an important role in maintaining *B. burgdorferi* or other *Borrelia* species in an enzootic cycle in rabbits in nearby southeastern Missouri and other areas of the eastern United States.24,33,44 Recent evidence of a possibly new *Borrelia* species from the lone star tick (*Amblyomma americanum*)45 and the variability of *B. burgdorferi* isolated from southeastern Missouri46 indicate the wide variety of *Borrelia* species or strains wild mammals are exposed to in the southeastern United States. Based on the serologic evidence, further research of the interactions and ecology among borreliial spirochetes, ticks, and hosts is necessary in Tennessee.

**Acknowledgments:** Appreciation is expressed to Paul Peel, Ted Pierce, Willie Crutcher, and Andrew Kuhn for their help in collecting ticks and sera from wild mammals. We also thank Zeus laboratories for providing ELISA titer plates.

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Human Infection With Tick-transmitted *Babesia microti* in Rhode Island: Serological Evidence and Risk Factor Assessment

Renjie Hu, PhD; Daniel Markowski, MS; Kerwin E. Hyland, PhD; Colleen Kelly, PhD; and Thomas N. Mather, PhD

**ABSTRACT**

**Background:** Human babesiosis, like Lyme disease, is transmitted principally by *Ixodes scapularis* in the United States. Babesiosis cases, however, have been reported far less frequently than Lyme disease, despite the same natural maintenance cycles of their causative pathogens.

**Methods:** Human sera were collected during 1994 and 1995 from Rhode Island residents and tested for anti-*Babesia microti* IgG antibodies using an indirect immunofluorescent antibody assay. A multiple logistic regression model was developed to assess predictive factors for the risk of acquiring the infection.

**Results:** Of 589 serum samples tested, 24 (4.1%) subjects exhibited titers ranging from 1:64 to 1:256. None had been diagnosed clinically. The prevalence of antibodies was significantly greater for subjects also seropositive for Lyme disease spirochetes, *Borrelia burgdorferi* (9.7%, n=154), than *B. burgdorferi* seronegative subjects (3.2%, n=185) (P<0.05). In addition, *B. burgdorferi* seroreactivity was the only significant risk predictor of *B. microti* infection (odds ratio [OR]=3.68, 95%; CI=2.71 to 4.65; relative risk [RR]=3.42); age and sex did not constitute significant risk factors of acquiring the infection.

**Conclusion:** This study suggests that people exposed to *B. burgdorferi* infection and frequent encounters with *I. scapularis* are at greater risk for infection with *B. microti*, and that within certain geographic regions of Rhode Island with abundant *I. scapularis* ticks, human infection with this pathogen is more common than previously thought.

**Key words:** *Ixodes scapularis*, *Babesia microti*, *Borrelia burgdorferi*, tick-borne disease, serological survey, risk factor

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**INTRODUCTION**

Infection with the tick-transmitted piroplasm *Babesia microti* is the most common cause of human babesiosis in the United States.1,2 Most infections are transmitted by the black-legged tick, *Ixodes scapularis*,3 which also is the vector of Lyme disease spirochetes, *Borrelia burgdorferi*.4 In endemic areas, white-footed mice (*Peromyscus leucopus*) serve as the principal reservoir hosts for both infections.5-8 Where both pathogens occur, coinfection in this rodent host is common.9 Consequently, *I. scapularis* can be infected concurrently and is capable of simultaneous transmission of both pathogens to susceptible hosts.10,11

Lyme disease is the most frequently reported arthropod-borne disease in the United States; human babesiosis cases appear far less commonly. In 1994, 13,083 Lyme disease cases from 43 states were reported to the Centers for Disease Control and Prevention.12 In comparison, only a few hundred cases of human babesiosis have been documented within the United States in recent years.13-16 Clinically, human babesiosis is a malaria-like disease, often presenting symptoms of fever, headache, fatigue, chills, malaise, and anemia.1,17 Symptoms usually are vague and infections self-limiting except in asplenic,
immunocompromised, or certain elderly patients.\textsuperscript{17-20} Most recognized human babesiosis cases have been clustered on several islands along the southern coastal region of New England and New York,\textsuperscript{7,13,14,21,22} where I. scapularis and P. leucopus occur together in abundance. More rarely, infections acquired by blood transfusion have been documented.\textsuperscript{23-26} The bite of an infective tick appears to be the main route of transmission to humans for B. microti and B. burgdorferi\textsuperscript{27,28} and coinfection in humans has been demonstrated serologically.\textsuperscript{29,30}

The relatively widespread distribution of the tick vector and the overlapping natural maintenance cycles of B. microti and B. burgdorferi suggest that many more B. microti infections should be expected to occur. Studies have demonstrated, however, that this piroplasm is transmitted less efficiently than Lyme disease spirochetes from host to vector,\textsuperscript{31} and that vector population densities required for natural maintenance of B. microti may need to be higher than those required for B. burgdorferi maintenance.\textsuperscript{32} Both factors help explain the disparity in the number of B. microti and B. burgdorferi infections. Human B. microti infection could be limited to subsets of areas within the geographical distribution of I. scapularis where these ticks attain the required minimum population density. Additionally, human infection with B. microti is perhaps more common within I. scapularis-endemic areas even though its symptomatic presentation is rare. Recent tick surveillance studies in Rhode Island have shown that I. scapularis densities throughout the state vary from nonexistent to hyperabundant.\textsuperscript{33} To get a better understanding of the risk factors that determine human infection with B. microti, we conducted a serological study of patient cohorts derived from areas where I. scapularis abundance was either high or low. In particular, we determined the prevalence of anti-B. microti IgG antibodies in serum samples submitted for Lyme disease testing and other reasons unrelated to tick-borne diseases, evaluated risk of exposure to babesial infection in I. scapularis abundant and rare areas, and assessed the significance of epidemiologic factors, including age and sex, for the risk of exposure to B. microti infection.

**MATERIALS AND METHODS**

**Source of sera**

Human serum samples used in this study were provided by three regional hospitals and the Rhode Island Department of Health Laboratories (DOHL). Sera originally were submitted to these laboratories either for serological diagnosis of Lyme disease or for other tests unrelated to tick-borne diseases. Participating serum providers were located within the portion of Rhode Island where I. scapularis is abundant (Westerly and South County Hospitals and DOHL) or outside of that area (Newport Hospital). Thus, sera presumably were collected from patients from either tick-abundant or rare areas. Reactivity to B. burgdorferi of sera submitted for the diagnosis of Lyme disease was determined by the individual laboratory to which they were submitted.

**Detection of anti-B. microti antibodies**

All sera were tested for anti-B. microti IgG antibodies by an indirect immunofluorescent antibody (IFA) assay procedure.\textsuperscript{20,34,35} To prepare B. microti antigen slides for use in the IFA, two 4-week-old, female, Syrian golden hamsters (Mesocricetus auratus) were inoculated intraperitoneally with the Webster strain of B. microti. This strain originally was derived from a hamster inoculated with a pool of blood taken from five P. leucopus captured live at the site of a 1992 fatal case of human babesiosis in Charlestown, RI. After isolation, aliquots of infected blood were diluted in an equal volume of 20% dimethyl sulfoxide (DMSO) and stored at -70°C. Hamsters used to make the antigen were inoculated intraperitoneally with 0.2 mL of this solution.

After inoculation, thin blood smears were prepared weekly and examined for parasitemia using a Giemsa staining procedure. Four weeks after inoculation, hamsters were anesthetized using halothane (Halocarbon Laboratories, North Augusta, SC) and 0.2 mL of blood was taken by cardiac puncture from each animal. The blood was mixed with ethylenediaminetetraacetic acid (EDTA) to prevent coagulation, and then centrifuged at 2000 rpm for 10 minutes to separate erythrocytes from plasma. Plasma was removed and erythrocytes were washed three times in 0.01 M phosphate buffered saline (PBS) (pH=7.6) by centrifugation at 2000 rpm for 10 minutes. After the final wash, erythrocytes were resuspended in 700 µL of PBS (to yield a concentration of approximately 5×10\textsuperscript{8} cells/mL) and thick smears were prepared on 8-well slides (Cel-line, Newfield, NJ). After air drying at room temperature, the slides were stored at -70°C until they were used 3 to 16 weeks later.

All human sera were diluted at 1:32 in 0.01 M PBS (pH=7.6), placed onto IFA slides, and incubated for 30 minutes at 37°C in a humid chamber. After incubation with patient sera, slides were washed in PBS for 10 minutes, air dried, and treated with 1:64 dilution of a goat antimouse immunoglobulin (IgG) conjugate labeled with fluorescein isothiocyanate (Sigma) and 0.01% Evans Blue added as a counterstain. Slides were reincubated at 37°C for 30 minutes, after which they were washed again, air dried, and examined by epifluorescence at ×400 magnification. Controls used in each run included known positive and negative human sera against B. microti and a PBS sham in place of human serum. All positive sera were
retested to determine their IgG titers. A dilution of 1:64 was considered the cutoff point for positives.

Statistical analysis of results

A Mantel-Haenszel test was used to detect the difference in anti- Babesia microti antibody prevalence in tick-abundant or rare areas. A Chi-square test was used to determine differences in the prevalence established for groups of B. burgdorferi positive and negative, sera collected for reasons not related to tick-borne diseases, and between males and females. In addition, a multiple logistic regression model was developed to evaluate risk factors associated with B. microti infection, including infection with B. burgdorferi, age, and sex. A backward stepwise method of elimination was used in the model to determine significant risk predictors of the infection.

RESULTS

A total of 589 serum samples collected during 1994 and 1995 were tested for anti-B. microti IgG antibodies by IFA. Overall, 24 (4.1%) patients exhibited detectable titers ranging from 1:64 to 1:512 (mode=1:64). The median age of patients sampled was 41 (range=2 to 93); the median age of individuals seropositive to B. microti was 45 (range=3 to 86 years). The highest seroprevalence was detected among the group older than 69 (6.7%, n=60); the lowest occurred among 20- to 29-year-olds (1.3%, n=80) (Fig). Seropositivity among males (4.3%, n=207) and females (3.3%, n=329) was not statistically different (χ²=0.36, P=0.55). Age and sex were unknown for 53 subjects.

Overall, the prevalence of anti-B. microti antibodies detected in serum samples from the I. scapularis abundant region (4.8%, n=505) was not significantly higher than that from tick-rare areas (0%, n=84) (M²=1.19; P=0.275) (Table). Within tick-abundant regions, the prevalence of anti-B. microti antibodies was significantly higher in patients whose samples were submitted for Lyme disease testing (6.2%, n=339) than in patients whose samples were submitted for tests unrelated to tick-borne diseases (1.8%, n=166) (χ²=4.74, P<0.05). Among those tested for Lyme disease, patients with sera reactive to B. burgdorferi exhibited significantly higher prevalence of anti-B. microti antibodies (9.7%, n=154) than those found to be B. burgdorferi negative (3.2%, n=185) (χ²=6.11, P<0.05).

A multiple logistic regression model that included information on B. microti seroreactivity, B. burgdorferi seroreactivity, age, and sex indicated that B. burgdorferi seroreactivity was the only significant risk factor contributing to B. microti infection (P<0.05). Neither patient age (P=0.43) nor sex (P=0.81) contributed significantly to the risk of acquiring B. microti infection. Following stepwise elimination of independent variables of age and sex from the model, an odds ratio (OR) and relative risk (RR) were calculated for the only remaining valid variable: seroreactivity to B. burgdorferi. The odds of exposure to babesial infection were nearly four times higher in B. burgdorferi seropositive patients than B. burgdorferi seronegative individuals (OR=3.68, 95% CI=2.71 to 4.65). In addition, the probability of exposure to B. microti infection was more than three times higher (RR=3.42) among B. burgdorferi seropositive patients than among those lacking evidence of exposure to spirochetes.

DISCUSSION

Human babesiosis caused by B. microti remains a relatively rare tick-borne disease despite being maintained and transmitted in the same enzootic cycle as Lyme disease spirochetes in the northeastern and upper midwestern
United States. Rhode Island has one of the highest Lyme disease incidence rates in the country (e.g., 46.9/100,000 in 1994). Since 1989, the incidence rate of babesiosis cases reported annually has averaged only about 0.1/100,000. Nevertheless, human infection with *B. microti* may be far more common than anticipated from case reports. Serological surveys conducted in the northeastern region have indicated that prevalence of the infection among selected cohorts ranges between 1.8% and 9.4%. In our study, 24 (4.1%) of the 589 Rhode Island residents tested for the presence of anti-*B. microti* IgG antibodies exhibited evidence of previous or current infection. Although none of these studies estimates the overall prevalence of *B. microti* infection in the population, they suggest that, at least in coastal New England, human babesial infections may occur more frequently than are diagnosed or reported.

Risk of exposure to *B. microti* will be greatest in regions where vector ticks are abundant. A study recently completed in Rhode Island demonstrated that zoonotic *B. microti* infection in white-footed mice was detected only among mice captured in areas where vector ticks were at least moderately abundant (>20 nymphal *I. scapularis*/hr flagging) and never in areas where tick populations were low or nonexistent. In the current study, we derived human serum samples from sources located in *I. scapularis*-abundant portions of the state (South County Hospital, Westerly Hospital, and DOHL) as well as from a region where these ticks rarely are present (Newport Hospital). Anti-*B. microti* antibodies were not detected from patients seeking medical attention in the tick-rare region (Newport, n=84). Apparently, risk of acquiring *B. microti* infection is greater among residents in regions where *I. scapularis* occurs abundantly than among those in areas where this tick rarely is present. Unfortunately, our sample size from the tick rare region would need to be more than twice the number actually collected to obtain statistical significance. Achieving such a sample size during the study period in areas where Lyme disease tests rarely are requested by physicians was impossible.

Our study indicated that an individual's age and sex were not significant risk factors for acquiring *B. microti* infection. Seroreactivity to *B. burgdorferi* was the only predictive risk factor of acquiring *B. microti* infection among independent variables tested; 9.7% of *B. burgdorferi* seropositive subjects also had evidence of *B. microti* infection. Similarly, high rates of anti-*B. microti* antibodies have been observed previously among patients with clinical Lyme disease or *B. burgdorferi* seropositives in New York and Connecticut. Because both *B. burgdorferi* and *B. microti* can be transmitted by *I. scapularis*, and these ticks may be infected with both pathogens, the observed positive association between *B. burgdorferi* and *B. microti* seropositivity may be related largely to coinfection events. This need not be the case, however, as infection with these pathogens can result from exposure to several infected *I. scapularis*. Most people with either infection are at greater risk of tick exposure and may have experienced multiple tick bites. That 3.2% of *B. burgdorferi*-seropositive people in our study exhibited anti-*B. microti* IgG antibodies supports the idea that human infection with *B. microti* can occur independently from infection with *B. burgdorferi*.

None of the patients in the current study was diagnosed clinically with babesiosis, and our purpose was not to distinguish active infections from those contracted previously. Studies have suggested that IgG levels in patients with active *B. microti* infection can be elevated above 1:1024 and patients with titers of around 1:64 probably have developed infection at least 6 months earlier. In our study, positive titers ranged between 1:64 and 1:256, suggesting that none was experiencing active infection. The few individuals (3.2%, n=185) whose sera were *B. burgdorferi* negative but *B. microti* seropositive may have been infected recently and possibly were in an early phase of anti-*B. burgdorferi* antibody development.

Clinical symptoms of babesiosis usually include fever, chills, fatigue, splenomegaly, and anemia. The gross similarity of these symptoms with those of Lyme disease and newly recognized human mononcytic and granulocytic ehrlichioses suggests that diagnosis of *B. microti* infection, if not overlooked, frequently may be confounded with other infections, especially in areas where vector ticks are abundant. The intensity of clinical manifestations caused by *B. microti* appears to be greater in adults older than 40 than in younger adults or children, although debilitating illness can occur among all age groups.

Studies to delineate the full spectrum of disease in *B. microti*-infected individuals have yet to be carried out. With the prospect of more severe disease expression in individuals coinfected with *B. microti* and *B. burgdorferi*, or coinfected with mononcytic or granulocytic *Ehrlichia*, physicians must consider the exposure of the patient to all of these infections when any of these tick-borne diseases is diagnosed or suspected.

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Guest Editorial

Surveillance of Tick-borne Disease Really Is Important

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With every issue, the Journal of Spirochetal and Tick-borne Diseases strives to educate health professionals about tick-borne disease. This special section focuses on tick-borne disease surveillance. We intend to inform readers about surveillance processes, illustrate the importance of disease reporting, and summarize epidemiologic features of Lyme disease and other tick-borne diseases throughout the United States.

The word "surveillance" refers to watching over someone or something to monitor or control activity. In public health, surveillance involves collecting and analyzing data used to identify variations in trends or disease distribution; detecting and responding to disease threats; identifying risk factors; and recommending and assessing intervention and prevention strategies. Another component of surveillance is providing feedback of information to health care providers and policy makers. The ultimate goal is to improve public health and welfare and reduce health care costs.

Surveillance data are acquired through disease reporting, over which the states have authority. Each state has its own regulations regarding reporting procedures and a list of notifiable conditions. Reports can be provider initiated (passive surveillance) or health department solicited (active surveillance). Either way, health care professionals are very important to the reporting process and must be cognizant of the benefits of reporting. After a case report has been received, public health officials often conduct case investigations to assess sources of exposure and implement specific control measures. These officials also try to provide timely feedback, supply physicians with knowledge necessary to make accurate diagnoses, and educate the public about the risks of disease.

Not all diseases are reportable. The list of notifiable conditions differs from state to state. At least one tick-borne disease, however, is notifiable in every state; a variety are reported each year. Some, including Colorado tick fever, Rocky Mountain spotted fever (RMSF), tick-borne relapsing fever (TBRF), and tularemia, were first identified many years ago. Newly recognized, emerging, or reemerging tick-borne infections include human monocytotropic ehrlichiosis, human granulocytotropic ehrlichiosis, Lyme disease, and possibly, babesiosis. By definition, emerging infections pose a public health threat. Although not as sinister as ebola or hantavirus pulmonary syndrome, tick-borne illnesses can be, and often are, very serious.

Over the years, analysis of surveillance data has shown a significant decrease in the incidence of TBRF and tularemia, although endemic foci remain. According to these data, RMSF cases occur nationwide—the disease is not limited to the Rocky Mountain states as the name implies. Likewise, Lyme disease cases regularly occur outside the northeastern United States. As epidemiologists gather data pertaining to ehrlichiosis and babesiosis, they will see similar trends and, in all probability, find evidence for previously undetected tick-borne infections.

Although the concepts of surveillance and reporting are fairly straightforward, difficulties are associated with surveillance, diagnosis, and reporting of tick-borne diseases. Surveillance capability varies across the United States. As mentioned, the list of notifiable tick-borne illnesses varies from state to state. As is the case with many infectious diseases, underreporting persists; tick-borne diseases such as RMSF may be underreported by at least a factor of four. To make matters worse, relatively little is known about the incidence and etiologic agents of rickettsial illnesses. Many health care providers have been told that Lyme disease does not occur or rarely occurs in most parts of the United States.

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States, so these diagnoses may not be considered. Varying strains of tick-borne bacteria, or concurrent infection with two or more organisms, may produce atypical illnesses that do not meet accepted case definitions. Even when tick-borne infections are considered, not many laboratory tests useful during the acute stage of illness when decisions regarding therapy must be made are available. Because of these problems, data concerning tick-borne diseases continue to be collected.

As you read the following surveillance articles, think about reporting. If you are not a designated “reporter,” remind someone who is about the importance of reporting. You may play a part in ensuring that a physician correctly diagnoses a case of Lyme disease, that a patient with ehrlichiosis receives an appropriate antibiotic, or that a research scientist gets a grant to study babesiosis.

REFERENCES
Case Definitions for Public Health Surveillance

INTRODUCTION

Public health officials rely on health providers, laboratories, and other public health personnel to report the occurrence of notifiable diseases to state and local health departments. Without such data, monitoring trends or evaluating the effectiveness of intervention activities would be difficult.

The Council of State and Territorial Epidemiologists (CSTE) has recommended that state health departments report cases of selected diseases to the National Notifiable Diseases Surveillance System (NNDSS) of the Centers for Disease Control and Prevention (CDC). The usefulness of such data, however, has been limited by the lack of uniform case definitions for public health surveillance. Without explicit criteria for identifying cases, state health departments and individual practitioners have used various criteria for case reporting. This document, prepared in cooperation with the CSTE, provides uniform criteria for reporting purposes. States that wish to improve the specificity of reporting may find the definitions helpful. As uniform case definitions are adopted, the incidence of reported diseases in different geographic areas may be compared more meaningfully.

In the United States, requirements for reporting diseases are mandated by state laws or regulations, and the list of reportable diseases in each state varies. A summary of state requirements for notifiable diseases recently has been published. National data from the NNDSS are collated and published weekly in the Morbidity and Mortality Weekly Report (MMWR). In general, cases reported by state health departments to the NNDSS are provisional. Updated final reports are published annually in the Summary of Notifiable Diseases.

Additionally, state health departments provide CDC information about these and other conditions of public health interest through supplementary surveillance systems that collect more detailed, condition-specific information. These conditions may or may not be included in the state laws or regulations that mandate reporting.

The CSTE/CDC surveillance case definitions included in this document vary in their use of clinical, laboratory, and epidemiologic criteria to define cases. Some clinical syndromes do not have confirmatory laboratory tests, but laboratory evidence may be one component of a clinical definition; toxic shock syndrome is an example. Other diseases (eg, mumps) have such a characteristic clinical presentation that, even in the absence of confirmatory laboratory testing, a diagnosis may be based only on clinical findings. In most instances, a brief clinical description is provided. Unless the clinical description is explicitly cited in the “Case classification” section of each definition, it is included only as background information.

Some diseases require laboratory confirmation for diagnosis, regardless of clinical symptomatology, and some are diagnosed on the basis of epidemiologic data. Many of the childhood vaccine-preventable diseases include epidemiologic criteria (eg, exposure to probable or confirmed cases of disease) in the case definitions. In some instances, the site of infection may be important; for example, pharyngeal diphtheria is notifiable, whereas cutaneous diphtheria is not.

For many diseases, substantial amounts of information, including results of laboratory tests, must be collected before a final case classification is possible. State health departments are requested to continue reporting provisional cases to the NNDSS promptly, and records should be updated when additional surveillance information becomes available.

Surveillance demands uniformity, simplicity, and brevity. These case definitions are intended to establish uniform criteria for disease reporting; they should not be used as sole criteria for establishing clinical diagnoses, determining the standard of care necessary for a particular patient, setting guidelines for quality assurance, providing standards for reimbursement, or initiating public health actions. Use of additional clinical, epidemiologic, and laboratory data may enable a physician to diagnose a disease even though the surveillance case definition may not be met. For example, an adolescent with bilateral orchitis who attends a school in which a mumps outbreak is occurring would not meet the surveillance case definition for mumps unless the mumps virus was isolated. Clinical judgment, however, would suggest that in this situation, viral isolation is not necessary.
As knowledge increases and diagnostic technology improves, some definitions will change to reflect those trends. For example, many cases of non-A, non-B hepatitis are due to the recently described hepatitis C virus. Therefore, revisions, additions, and deletions can be expected in the future.

**DEFINITION OF TERMS USED IN CASE CLASSIFICATION**

**Confirmed case**: a case classified as confirmed for reporting purposes.

**Probable case**: a case classified as probable for reporting purposes.

**Laboratory-confirmed case**: a case confirmed by one or more of the laboratory methods listed in the case definition under “Laboratory criteria for diagnosis.” Although other laboratory methods may be used in clinical diagnosis, only those listed are accepted for laboratory confirmation for reporting purposes.

**Clinically compatible case**: a clinical syndrome generally compatible with the disease, but no specific clinical criteria must be met unless noted in the case classification.

**Supportive laboratory results**: specified laboratory results consistent with the diagnosis but not meeting the criteria for laboratory confirmation.

**Epidemiologically linked case**: a case in which the patient has or has had contact with one or more persons who have or have had the disease, and transmission of the agent by the usual modes of transmission is plausible. A case may be considered epidemiologically linked to a laboratory-confirmed case if at least one case in the chain of transmission is laboratory confirmed.

**Meets the clinical case definition**: meets precisely the clinical case definition. Although in clinical practice the diagnosis may be made with the use of other criteria, for reporting purposes the stated criteria must be met.

**CASE DEFINITIONS**

**Lyme Disease**

**Clinical description**

A systemic, tick-borne disease with protean manifestations, including dermatologic, rheumatologic, neurologic, and cardiac abnormalities. The best clinical marker for the disease is the initial skin lesion, erythema migrans (EM), that occurs among 60% to 80% of patients.

**Clinical case definition**

- EM, or
- at least one late manifestation, as defined in the following, and laboratory confirmation of infection.

**Laboratory criteria for diagnosis**

- Isolation of *Borrelia burgdorferi* from clinical specimen,
- demonstration of diagnostic levels of IgM and IgG antibodies to the spirochete in serum or CSF, or
- significant change in IgM or IgG antibody response to *B. burgdorferi* in paired acute- and convalescent-phase serum samples.

**Case classification**

Confirmed: a case that meets one of the clinical case definitions listed previously.

**Comment**

This surveillance case definition was developed for national reporting of Lyme disease; it is NOT appropriate for clinical diagnosis.

Definition of terms used in the clinical description and case definition:

**A. EM.** For purposes of surveillance, EM is defined as a skin lesion that typically begins as a red macule or papule and expands over a period of days to weeks to form a large round lesion, often with partial central clearing. A solitary lesion must reach at least 5 cm in size. Secondary lesions also may occur. Annular erythematous lesions occurring within several hours of a tick bite represent hypersensitivity reactions and do not qualify as EM. For most patients, the expanding EM lesion is accompanied by other acute symptoms, particularly fatigue, fever, headache, mild stiff neck, arthralgia, or myalgia. These symptoms typically are intermittent. The diagnosis of EM must be made by a physician. Laboratory confirmation is recommended for persons with no known exposure.

**B. Late manifestations.** Late manifestations include any of the following when an alternate explanation is not found:

- Musculoskeletal system; recurrent, brief attacks (weeks or months) of objective joint swelling in one or a few joints, sometimes followed by chronic arthritis in one or a few joints. Manifestations not considered as criteria for diagnosis include chronic progressive arthritis not preceded by brief attacks and chronic symmetrical polyarthritis. Arthralgia, myalgia, or fibromyalgia syndromes alone are not criteria for musculoskeletal involvement.
- Nervous system; any of the following, alone or in combination: lymphocytic meningitis; cranial neuritis, particularly facial palsy (may be bilateral); radiculoneuropathy; or, rarely, encephalomyelitis. Encephalomyelitis must be confirmed by showing antibody production against *B. burgdorferi* in the cerebrospinal fluid (CSF), demonstrated by a higher titer of antibody in CSF than in serum. Headache, fatigue, paresthesia, or mild stiff neck alone are not criteria for neurologic involvement.


- Cardiovascular system; acute onset, high-grade (2nd or 3rd degree) atrioventricular conduction defects that resolve in days to weeks and are sometimes associated with myocarditis. Palpitations, bradycardia, bundle branch block, or myocarditis alone are not criteria for cardiovascular involvement.

C. Exposure. Exposure is defined as having been in wooded, brushy, or grassy areas (potential tick habitats) in a county in which Lyme disease is endemic no more than 30 days before EM onset. A history of tick bite is NOT required.

D. Disease endemic to county. A county in which Lyme disease is endemic is one in which at least two definite cases have been acquired previously or in which a known tick vector has been shown to be infected with *B. burgdorferi*.

E. Laboratory confirmation. As noted, laboratory confirmation of infection with *B. burgdorferi* is established when a laboratory isolates the spirochete from tissue or body fluid, detects diagnostic levels of IgM or IgG antibodies to the spirochete in serum or CSF, or detects a significant change in antibody levels in paired acute- and convalescent-phase serum samples. States may determine the criteria for laboratory confirmation and diagnostic levels of antibody. Syphilis and other known causes of biologic false-positive serologic test results should be excluded when laboratory confirmation has been based on serologic testing alone.

Rocky Mountain Spotted Fever

Clinical description
An illness most commonly characterized by acute onset and fever, usually accompanied by myalgia, headache, and petechial rash (on the palms and soles in two thirds of the cases)

Laboratory criteria for diagnosis
- Fourfold or greater rise in antibody titer to the spotted fever group antigen by immunofluorescent antibody (IFA), complement fixation (CF), latex agglutination (LA), microagglutination (MA), or indirect hemagglutination (IHA), or a single titer ≥64 by IFA or ≥16 by CF.
- Demonstration of positive immunofluorescence of skin lesion (biopsy) or organ tissue (autopsy).
- Isolation of *Rickettsia rickettsii* from clinical specimen.

Case classification
Probable: a clinically compatible case with supportive serology (fourfold rise in titer or a single titer ≥320 by Proteus OX-19 or OX-2, or a single titer ≥128 by LA, IHA, or MA test).
Confirmed: a case that is laboratory confirmed.

**Tularemia**

Clinical description
An illness characterized by several distinct forms, including:
- Ulceroglandular—cutaneous ulcer with regional lymphadenopathy.
- Glandular—regional lymphadenopathy with no ulcer.
- Oculoglandular—conjunctivitis with preauricular lymphadenopathy.
- Intestinal—pharyngitis, intestinal pain, vomiting, and diarrhea.
- Pneumonic—primary pleuropulmonary disease.
- Typhoidal—febrile illness without early localizing signs and symptoms.

Clinical diagnosis is supported by evidence or history of a tick or deerfly bite, exposure to tissues of a mammalian host of *Francisella tularensis*, or exposure to potentially contaminated water.

Laboratory criteria for diagnosis
- Isolation of *F. tularensis* from a clinical specimen;
- Demonstration of *F. tularensis* in a clinical specimen by immunofluorescence; or
- Fourfold or greater rise in agglutination titer between acute- and convalescent-phase serum specimens obtained ≥2 weeks apart, analyzed at the same time and in the same laboratory.

Case classification
Probable: a clinically compatible case with supportive serologic results (tularemia agglutination titer of ≥160 in one or more serum specimens obtained after onset of symptoms).
Confirmed: a case that is laboratory confirmed.

**Babesiosis**

Identification
A potentially severe and sometimes fatal disease caused by infection with a protozoan parasite of RBCs. The clinical syndrome may include fever, chills, myalgia, fatigue, and jaundice secondary to a hemolytic anemia and may last from several days to a few months. Asymptomatic infections occur, but their proportion is not known. Cases caused by parasite species found in Europe are more likely to be severe or fatal than those caused by the species prevalent in the United States. Dual infection with *B. burgdorferi* is known to occur.
Diagnosis is made by identification of the parasite within red blood cells on a thick or thin blood film. Demonstration of specific antibodies by serologic analysis (IFA) and isolation of the parasite in appropriate laboratory animals provide supportive evidence for the diagnosis. Differentiation from *Plasmodium falciparum* on blood film examination may be difficult in patients who have been in malarious areas or who may have acquired infection by blood transfusion; if diagnosis is uncertain, manage as if it were a case of malaria and send thick and thin blood films to an appropriate reference laboratory.

**Infectious agents**

*Babesia microti* and other *Babesia* spp., especially *B. divergens* in Europe.

**Occurrence**

In the United States, the geographic distribution of *B. microti* infection has increased along with the widening range of the tick vector, *I. scapularis* (formerly *I. dammini*). Babesiosis is endemic on Nantucket and other islands in Massachusetts, Block Island, Shelter Island, eastern Long Island, and southeastern Connecticut. Infection also has been reported from Wisconsin and perhaps Minnesota. Human cases due to species other than *B. microti* have been identified in the United States (California and Washington state) and Mexico. In Europe, human infections caused by *B. divergens* have been reported from Belgium, France, Ireland, Scotland, Spain, Sweden, Russia, and Yugoslavia.

**Reservoir**

Rodents for *B. microti* and cattle for *B. divergens*.

**Mode of transmission**

*B. microti* is transmitted during the summer months by the bite of nymphal Ixodes ticks (*I. scapularis*) that had fed on infected deer mice (*Peromyscus leucopus*) and some other small mammals (eg, voles, *Microtus pennsylvaniae*).

The adult tick normally is found on deer (which are not infected by the parasite) but also may parasitize and be spread by a variety of mammalian and avian hosts.

The vector of *B. divergens* in Europe appears to be *I. ricinus*.

Occasionally, cases of babesiosis have been reported to have been transmitted by blood transfusion from asymptomatic but parasitemic donors. Patients usually do not recall a tick bite.

**Incubation period**

Variable; 1 week to 12 months has been reported.

**Period of communicability**

Not transmitted from person to person except by blood transfusion. Asymptomatic blood donors have been shown to be infectious for as long as 12 months after initial infection.

**Susceptibility and resistance**

Susceptibility to *B. microti* is assumed to be universal; immunocompromised, asplenic, and elderly persons are at a particular risk of symptomatic infection.


**Methods of control**

For details on methods of control, consult the American Public Health Association’s *Control of Communicable Diseases Manual.*

**Ehrlichiosis**

**Identification**

An acute, febrile, bacterial illness caused by a group of small pleomorphic organisms that survive in the phagosomes of mononuclear or polymorphonuclear leukocytes of the infected host. The organisms sometimes are observed within these cells.

Sennetsu fever, so far documented convincingly only in Japan, is characterized by sudden onset with fever, chills, general malaise, headache, muscle and joint pain, sore throat, and sleeplessness. Generalized lymphadenopathy with tenderness of the enlarged nodes is common. Lymphocytosis with postauricular and posterior cervical lymphadenopathy is similar to that seen in infectious mononucleosis. The disease course usually is benign; fatal cases have not been reported.

Human ehrlichiosis in the United States is a newly recognized disease. The spectrum of disease ranges from an illness so mild that no medical care is sought to a severe, life-threatening, or fatal disease. Symptoms usually are nonspecific; the most common complaints are fever, headache, anorexia, nausea, myalgia, and vomiting. The disease may be confused clinically with RMSF but differs by rarity of a prominent rash. Laboratory findings include leukopenia, thrombocytopenia, and elevation of one or more liver function tests. In hospitalized cases, the laboratory findings may be slightly abnormal on admission and become more abnormal during hospitalization.

Differential diagnosis includes RMSF, Lyme disease, toxic shock syndrome, and other multi-system febrile illnesses. The clinical diagnosis of sennetsu fever is confirmed by IF tests, using the etiologic agent isolated in
macrophage cultures. Diagnosis of ehrlichiosis in the United States is based on clinical and laboratory findings and the development of antibodies to *Ehrlichia chaffeensis* in the IF test, using an antigen derived from a human isolate. Occasionally, inclusions typical of *Ehrlichia* may be observed in the cytoplasm of circulating monocytes or granulocytes.

**Infectious agents**

*Ehrlichia sennetsu* is the etiologic agent of sennetsu fever. These organisms are members of the genus *Ehrlichia*, tribe Ehrlichiae, and family Rickettsiaceae; until 1984, they were classified as members of the genus *Rickettsia*. The causative agent of the majority of human cases of ehrlichiosis found in the United States is *E. chaffeensis* (the first human isolate was from a patient in Fort Chaffee, Arkansas). Recently, human cases have been reported to be associated with infection by an organism closely related to *E. phagocytophilia* or a closely related species causing human granulocytic ehrlichiosis (HGE).

**Occurrence**

Sennetsu fever appears to be confined to western Japan. Ehrlichiosis in North America has been concentrated in the southeastern and south central areas of the United States; more than 320 cases with 9 deaths have been identified through laboratory-based surveillance from 1987 through 1993. More than a dozen human cases, including 3 deaths, caused by a granulocytic ehrlichia related to *E. phagocytophilia* have occurred in northern Minnesota, Wisconsin, Connecticut, Maryland, and Florida.

**Reservoir**

Not known for either sennetsu fever or American ehrlichiosis.

**Mode of transmission**

Not known for sennetsu fever, although patients with the disease frequently are reported to have visited rivers or swampy areas near rivers within 3 to 4 weeks of onset. Ticks, probably *Amblyomma americanum*, are the vectors of American monocyctic ehrlichiosis; most patients report a tick bite or an association with wooded, tick-infested areas several weeks prior to onset of illness. Ehrlichiosis caused by the *E. phagocytophilia*-related organism in the northern Midwest and eastern seaboard may be vectored by *I. scapularis* ticks.

**Incubation period**

Fourteen days for sennetsu fever; 7 to 21 days for American ehrlichiosis.

**Period of communicability**

No evidence of transmission from person to person.

**Susceptibility and resistance**

Susceptibility is believed to be general. No data are available on protective immunity in humans from infections caused by these organisms.


**Methods of control**

For details on methods of control, consult the American Public Health Association's *Control of Communicable Diseases Manual*.

REFERENCES

Lyme Disease and Other Tick-borne Diseases in Massachusetts

Silvia M. Kreindel, DVM, MPH; Bela T. Matyas, MD; Michael W. McGuill, DVM; Timothy P. Broadbent, BS; and Alfred DeMaria, Jr, MD

ABSTRACT

OBJECTIVES: To describe Lyme disease and other tick-borne diseases in Massachusetts in an effort to assess incidence, identify risk factors, and develop and implement prevention and control measures.

METHODS: Data were obtained through passive surveillance involving local boards of health and physician and laboratory reporting for cases of Lyme disease, Rocky Mountain spotted fever (RMSF), babesiosis, and human granulocytic ehrlichiosis (HGE) cases reported to the Massachusetts Department of Public Health from 1988 through 1995.

RESULTS: The annual incidence rate for reported Lyme disease remained relatively stable from 1988 to 1990 (1.3 to 2.2 cases per 100 000 population), peaked during 1991 (4.4 cases per 100 000 population), and ranged between 2.5 and 4.1 cases per 100 000 population thereafter. Sixty-four percent of the reported cases had onset of Lyme disease between May and August. Data from 1993 through 1995 show that approximately 45% of cases included reports of erythema migrans (EM). Children ranging in age from 5 to 9 accounted for 9% of reported cases for 1988 through 1995 and had the highest age-specific cumulative incidence at 33.7 cases per 100 000 population. Adults from 45 to 49 had a cumulative incidence of 3.0 cases per 100 000 population for this time period.

For babesiosis and RMSF, reported annual incidences were relatively stable and low over the period 1988 to 1995 (0.01 to 0.24 cases per 100 000 population for each). Adults aged 35 to 39 and 30 to 34 had the highest cumulative incidence rates for reported babesiosis and RMSF, respectively.

DISCUSSION AND CONCLUSIONS: Lyme disease, babesiosis, and RMSF cases are reported regularly in Massachusetts. HGE cases among Massachusetts residents also have been reported in the literature. Lyme disease represents the most prevalent tick-borne disease in the state. Although the distributions of reported cases of these tick-borne diseases are not uniform throughout the state, and the exposure risks for these diseases are believed to be predominantly regional, cases have been reported statewide, particularly for Lyme disease. As a consequence of the state’s current passive surveillance system and other factors, tick-borne diseases are believed to be underreported in Massachusetts. Active surveillance may be necessary to document the frequency and patterns of tick-borne diseases in the state accurately. Implementation of recently developed serodiagnostic testing recommended by the Centers for Disease Control and Prevention is being encouraged to avoid misclassification of cases.

KEY WORDS: Lyme disease, babesiosis, Rocky Mountain spotted fever, passive surveillance

BACKGROUND

Lyme disease is the most commonly reported tick-borne disease in the United States. In 1994, 13 083 human cases of Lyme disease were reported to the US Centers for Disease Control and Prevention (CDC). Six northeastern states largely accounted for a 95% increase in reported Lyme disease cases for 1994. In Massachusetts, the deer tick, Ixodes scapularis, is

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believed to be the common vector of Lyme disease, babesiosis, and human granulocytic ehrlichiosis (HGE); Rocky Mountain spotted fever (RMSF) generally is transmitted by the dog tick, *Dermacentor variabilis*. In 1985, Lyme disease and babesiosis were made reportable in Massachusetts; RMSF was made reportable in the early 1950s. Although HGE is not yet reportable in the state, voluntary reporting to the Massachusetts Department of Public Health (MDPH) is encouraged.

Efforts to improve public health surveillance of tick-borne diseases date to the 1950s with the requirement for reporting RMSF. Lyme disease has been recognized in Massachusetts since the late 1970s. Not surprisingly, changes in case definitions and other surveillance measures as well as changes in vector and pathogen prevalence over time affect the number of reported cases. Tick-borne disease surveillance in Massachusetts is passive in nature, involving a combination of laboratory-based reporting where applicable and reporting by physicians to local boards of health (which, in turn, report to the MDPH). Some factors associated with underreporting by physicians include lack of knowledge of the reporting requirements or mechanisms, low priority among activities, and lack of familiarity with the diseases in non-coastal Massachusetts areas.

**METHODS**

Data for tick-borne disease cases were obtained from the MDPH communicable disease surveillance system. Local boards of health collect case reports through physician reporting, the receipt of laboratory reports, or another board of health. These case reports are then forwarded to the MDPH. A follow-up form for each case is sent to the board of health that reported the case or to the board of health where the patient with a positive serodiagnosis resides (in the case of laboratory reports sent directly to the MDPH). The original case reports and follow-up reports are recorded by the MDPH in the surveillance database. A more extensive surveillance data set has been available at the MDPH since 1993. Lyme disease, babesiosis, and RMSF cases that meet the CDC surveillance case definition criteria are counted as cases.

Cases are defined as Massachusetts residents reported to the MDPH and subsequently confirmed to meet the CDC case definition for Lyme disease (*n* = 1389), babesiosis (*n* = 63), or RMSF (*n* = 39) during the period from 1988 to 1995. Demographic variables were analyzed for the 1988 to 1995 period. Information on variables such as geographic location where the exposure could have occurred and early and late manifestations of Lyme disease were analyzed for the 1993 to 1995 period. Incidence rates were based on the 1990 US Census. Descriptive statistics were generated using Epi Info 6.02. Univariate analyses (*χ²* and *t* tests) were used to assess associations.

**RESULTS**

**Cumulative data**

Lyme disease: Of 1986 reports of Lyme disease received by the MDPH from 1988 to 1995, 1389 (70%) met the CDC’s surveillance case definition and were included in this analysis. Most (61%) of these case reports were received from local boards of health. Children from 5 to 9 had the highest rate of Lyme disease, with a cumulative incidence of 33.7 cases per 100,000 population for the time period from 1988 to 1995, representing 9% of reported cases. Adults aged 45 through 49 had the second highest rate, with a cumulative incidence of 30.0 cases per 100,000 population for the time period, representing 7.4% of the reported cases.

For the period from 1988 to 1995, 64% of the reported cases had onset of symptoms during the months of May through August. Nantucket, Barnstable, and Dukes counties (these counties represent Cape Cod and the Islands) represented the likely county of exposure for 56% of the cases reported from 1993 to 1995, with Nantucket County the most likely county of exposure (35% of cases).

Forty-five percent of Lyme disease case reports for the 1993 to 1995 period indicated the presence of *Ehrlichia migrans* (EM). Of the cases reporting EM, 35% noted arthritic manifestations, 9% noted neurologic manifestations, and 4% noted cardiologyc manifestations. Of the 235 cases not reporting EM, arthritic manifestations occurred in 77%, neurologic manifestations in 20%, and cardiologyc manifestations in 2%.

Babesiosis: Of the 67 reports of babesiosis from 1988 through 1995, 63 met the CDC’s surveillance case definition and were included in this analysis. Adults from 35 to 39 had the highest age-specific cumulative incidence at 1.66 cases per 100,000 population for the time period 1988 to 1995, and comprised 14% of reported CDC-eligible cases. Most (89%) of these reported cases resided in Nantucket County. Cases were reported most frequently during the fall (43%).

RMSF: Of the 70 reports of RMSF received from 1988 through 1995, 39 met the CDC’s surveillance case definition and were included in this analysis. Adults aged 30 to 34 represented 15% of the reported CDC-eligible cases from 1988 to 1995. Barnstable County reported the highest cumulative incidence of RMSF at 4.3 cases per 100,000 population for this time period, representing 21% of reported CDC-eligible cases for 1988 to 1995. A majority (64%) of cases were reported during the summer months, with remaining cases reported during the fall months.
Temporal trends

Fig 1 illustrates the number of cases of tick-borne disease reported to the MDPH from 1988 through 1995. For Lyme disease, the annual incidence rates remained relatively stable from 1988 to 1990 (1.3 to 2.2 cases per 100,000 population), peaking during 1991 (4.4 cases per 100,000 population), coincident with the change in the CDC’s surveillance case definition and ranging between 2.5 and 4.1 cases per 100,000 population thereafter (Figs 1, 2).

Fig 3 illustrates the age distribution of cases of Lyme disease reported from 1988 to 1995 in relation to the age distribution for Massachusetts.

During the 8 years evaluated, 64% of the reported cases of Lyme disease had onset during the months from May through August. Most cases were reported either during the months of July through October, reflecting typical delays in reporting of cases in a passive surveillance system, or in December, reflecting an end of the calendar year “catch up” artifact for disease reporting (Fig 4).

From 1993 to 1995, 45% of case reports indicated the presence of EM. No significant trends in these 3 years in the reporting of early or late Lyme disease manifestations were noted (Fig 5).

For babesiosis and RMSF, annual incidence rates for reported cases were relatively stable and low over the period from 1988 to 1995 (0.01 to 0.24 cases per 100,000 population for each). The 1995 incidence rate for babesiosis was 0.21 cases per 100,000 population, and the rate for RMSF was 0.01 cases per 100,000 population.

DISCUSSION AND CONCLUSIONS

Lyme disease, babesiosis, and RMSF cases are reported in Massachusetts regularly. HGE cases among Massachusetts residents also have been reported in the literature. Although the distributions of reported cases of these tick-borne diseases are not uniform throughout the state, and the exposure risks for these diseases are believed to be predominantly regional, cases have been reported statewide, particularly for Lyme disease.

Lyme disease represents the most prevalent tick-borne disease in Massachusetts. Over the past decade, Lyme dis-
ease has spread throughout the state, with major foci on Cape Cod and the islands of Nantucket and Martha's Vineyard and smaller foci in North Shore communities and in areas of western Massachusetts. Cases of Lyme disease have been confirmed in individuals with presumptive exposure in all counties in the state. Nantucket has reported the largest number of Lyme disease cases in Massachusetts and had the highest incidence of Lyme disease by county in the United States in 1994.

The data for Lyme disease from 1988 to 1995 (Figs 1, 2) are confounded by a significant change in the CDC surveillance case definition for Lyme disease in 1991. Most of the increase in the incidence between 1990 and 1991 may be attributable to the change in case definition. The year-to-year variation in reported cases of Lyme disease for 1991 to 1995 parallels changes in climatic factors (e.g., hot, humid summers) that might affect potential exposures to deer ticks. Variations in reporting behavior over this time period, however, cannot be assessed and, given the small numbers of reported cases, could be significant.

The age distribution for Lyme disease cases (Fig 3) is similar to the age distribution for Massachusetts residents, except for a peak in Lyme disease cases among children from 5 to 14 and another peak in cases among middle-aged adults. This age distribution of cases likely reflects exposure risks due to age-specific behaviors.

Annual variations in reported cases and incidence rates for babesiosis and RMSF are even more difficult to interpret. Reporting-related artifacts may have more significant effects on the reported incidence rates for these two diseases, given their smaller numbers. Insufficient awareness of the clinical presentations of babesiosis and RMSF by many primary care providers is a major factor in underreporting for these diseases. This factor can be addressed through targeted education for providers.

HGE is not a reportable disease in Massachusetts. Voluntary reporting of HGE is encouraged, but few case reports have been received by the MDPH to date. Given that the deer tick serves as a shared vector for Lyme disease, babesiosis, and HGE in Massachusetts, and given the prevalence of exposure to deer ticks in the state, both babesiosis and HGE may be more prevalent than is indicated by reporting of these diseases. Insufficient provider awareness of the clinical presentation of HGE is likely in view of its recent identification as a tick-borne disease.

The MDPH surveillance system is based on passive surveillance for tick-borne diseases and involves a combination of physician reports to local boards of health and laboratory reports of appropriate indicators to local boards of health or to the MDPH, with follow-up with health care providers. A passive surveillance system underestimates actual disease incidence for a variety of reasons. With tick-borne diseases, local prevalence of disease, geographic dependence of exposure risk recognition, and relatively low incidence complicate diagnosis and result in a nonuniform pattern of tick-borne disease recognition and reporting that does not reflect the underlying epidemiology of exposure risk. In combination with other factors inherent in passive surveillance, this may result in underreporting of tick-borne diseases in Massachusetts.

Active surveillance involving a combination of seasonal vector (exposure risk) surveillance, training of sentinel physicians, and targeted serosurveys might be necessary to document accurate incidence and geographic patterns of tick-borne diseases in Massachusetts. The adoption of new CDC recommendations for serologic testing for Lyme disease involving a two-step process (enzyme immunoassay or immunofluorescent assay followed by Western blot for confirmation) is being encouraged by the MDPH to help decrease problems associated with misclassification of cases.

REFERENCES

Lyme Disease Surveillance in Delaware, 1990 to 1994

David Wolfe, RN, MPH; Luis Callejas, MD, MPH; and Leroy Hathcock, PhD

ABSTRACT

Background: Since 1989, when Lyme disease became reportable in Delaware, 434 cases have been confirmed. Tularemia and Rocky Mountain spotted fever rarely are reported. Delaware's disease reporting system is passive. Hospital and private laboratories report the majority of cases. This study was conducted to describe the temporal, geographic, and demographic characteristics of Lyme disease in Delaware and to assist health planners in developing and implementing control strategies.

Methods: All physician-submitted Centers for Disease Control and Prevention (CDC) follow-up Lyme disease report forms from 1990 through 1994 were reviewed for completeness. Data were gathered only from completed forms. All cases were classified according to the 1990 CDC surveillance case definition.

Results: Reported cases of Lyme disease increased 113% between 1990 and 1994. The 1994 statewide incidence rate was 16.7 cases per 100,000 population. During the study period, whites were three times more likely to contract Lyme disease than were blacks. The majority of cases were reported between May and September.

Conclusion: The Delaware State Board of Health made Lyme disease reportable in September 1989. This requirement increased the quality of Lyme disease surveillance; however, the disease probably is underreported, as Delaware does not actively solicit Lyme disease reports. Delaware's case data reflect national data that indicate an increase in reported cases.

Key words: Lyme disease, Borrelia burgdorferi, Ixodes scapularis, surveillance, erythema migrans

BACKGROUND

Delaware requires the reporting of three tick-borne diseases: Lyme disease, Rocky Mountain spotted fever (RMSF), and tularemia. RMSF and tularemia have been reportable since the mid-1970s, and the Delaware State Board of Health made Lyme disease reportable in September, 1989. Since then, 434 cases of Lyme disease have been confirmed. During 1994, 111 cases of Lyme disease were confirmed, yielding a statewide attack rate of 16.7 cases per 100,000 population.

During Delaware's November, 1988 deer season, the University of Delaware, the Delaware Department of Natural Resources and Environmental Control, and the Delaware Division of Public Health tested ticks found on harvested white-tailed deer for the presence of Borrelia burgdorferi. Ixodes scapularis was the only tick species harboring the spirochete.

Fig. 1 depicts Delaware's communicable disease surveillance system. Reports are not solicited actively; therefore, the reporting system is passive. Physicians, laboratories, and hospitals report Lyme disease to the Delaware Division of Public Health through a mail-in postcard-sized disease report card. The Epidemiology Branch follows up the report by mailing a standard Centers for Disease Control and Prevention (CDC) Lyme disease case report form to the attending physician. The physician completes the form and returns it to the Epidemiology Branch. The CDC report form is then reviewed, and the case is classi-
fied as either confirmed or unconfirmed based on the October 1990 CDC/Council of State and Territorial Epidemiologists (CSTE) case definition.

Data gathered from these forms were used to prepare this article, which describes the temporal, geographic, and demographic characteristics of Lyme disease in Delaware.

METHODS

All physician-submitted CDC follow-up Lyme disease report forms from 1990 through 1994 were reviewed. Data about RMSF was gathered only from the disease report cards. Further characterization of RMSF and tularemia cases was not performed as few cases are reported in Delaware. Data used to prepare Table 1 (percent Lyme disease cases by source of report) and RMSF data (Fig 2) were gathered from the disease report cards; all other Lyme disease data was gathered from the CDC report forms.

A Lyme disease case was considered confirmed if it met one of the following criteria: The patient had erythema migrans (EM), as noted by the physician on the report form; or a patient had a positive serologic test and at least one rheumatologic, neurologic, or cardiologic manifestation.

RESULTS

Confirmed cases of Lyme disease and RMSF reported from 1990 through 1994 are shown in Fig 2. Between 1990 and 1994, the number of confirmed cases of Lyme disease has more than doubled. No confirmed cases of tularemia, ehrlichiosis, babesiosis, relapsing fever, or Colorado tick fever were reported between 1990 and 1994.

Lyme disease cases by month of onset are depicted in Fig 3. The majority of Lyme disease cases are reported between May and September.

Fig 4 summarizes Lyme disease at the county level. New Castle County had consistently higher rates per 100,000 population than did Kent or Sussex counties. New Castle County’s rate increased 125%, from 9.72 cases per 100,000 population in 1990 to 21.9 cases per 100,000 population in 1994.
100,000 population in 1994. Kent County’s rate increased 16%, from 4.5 cases per 100,000 population in 1990 to 6.3 cases per 100,000 population in 1994. Sussex County’s rate increased 138% between 1990 and 1994.

Fig 5 summarizes case rates by 10-year age group. Age-specific incidence rates were highest among the age group 50 to 59.

Fig 6 summarizes the case rates per 100,000 population by race. The case rate for whites increased 115%, from 8.3 cases per 100,000 population in 1990 to 17.9 cases per 100,000 population in 1994. The case rate for blacks showed little variation, averaging 4.2 cases per 100,000 population. On average, whites were three times more likely to contract Lyme disease than were blacks.

Table 1 summarizes Lyme disease cases by source of report. Private laboratories reported the majority (30% to 74%) of Lyme disease cases in Delaware.

Table 2 summarizes Lyme disease signs and symptoms as reported by Delaware physicians. Delaware physicians noted erythema migrans (EM) as a sign on average in 50% of their patients. The most common neurologic sign was Bell’s palsy. Cardiac signs rarely were observed.

Fig 7 depicts the date of onset of patients who demonstrated EM at diagnosis. The majority of cases developed EM between May and September of each year.

During 1990 and 1991, females were at slightly more risk of contracting Lyme disease than were males. This trend was reversed in 1992. Fig 8 represents the distribution of cases by sex.

Table 3 summarizes the location of exposure. Most (52% to 75%) of Delaware’s patients with Lyme disease believed they were exposed in Delaware. Over a 5-year period, an average of 8% (2% to 17%) feel they were exposed in another state, and 24% (15% to 31%) did not know where they were exposed to Lyme disease.

**DISCUSSION**

Prior to 1989, reporting was voluntary, and only 19 cases were reported. Delaware’s Lyme disease cases have doubled since 1990. Several factors have contributed to this increase: a standardized case definition, increased physician and public awareness, increased laboratory testing, required reporting, and an increase in the number of cases. Underreporting, however, is a major limitation of passive surveillance systems such as the
physician/laboratory-based postcard system now used in Delaware.² For the more common outpatient diseases, only 10% to 25% of the actual number of cases occurring in a community may be reported.²³ Therefore, even though case counts have increased since 1990, these counts probably underestimate the true incidence of Lyme disease in the state.

Between 1990 and 1994, whites were three times more likely to contract Lyme disease than were blacks. Factors that may contribute to this disparity include frequency of outdoor activity, tick feeding preferences, living location (suburban versus urban), susceptibility to the disease, and accessibility of medical care.

### Table 3
**Delaware Lyme Disease, Percent of Cases by Source of Exposure, 1990 to 1994**

<table>
<thead>
<tr>
<th>Year</th>
<th>In state</th>
<th>Unknown</th>
<th>Out of state</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>52% (27)</td>
<td>31% (16)</td>
<td>17% (9)</td>
<td>52</td>
</tr>
<tr>
<td>1991</td>
<td>68% (59)</td>
<td>25% (22)</td>
<td>7% (6)</td>
<td>87</td>
</tr>
<tr>
<td>1992</td>
<td>75% (63)</td>
<td>15% (13)</td>
<td>10% (8)</td>
<td>84</td>
</tr>
<tr>
<td>1993</td>
<td>72% (59)</td>
<td>24% (20)</td>
<td>4% (3)</td>
<td>82</td>
</tr>
<tr>
<td>1994</td>
<td>71% (79)</td>
<td>27% (30)</td>
<td>2% (2)</td>
<td>111</td>
</tr>
</tbody>
</table>

The age distribution of Lyme disease cases in Delaware reflects national trends. Most Lyme disease cases occur from the ages of 1 to 19 and 40 to 69 in the United States.⁵ EM is present in 50% to 85% of Lyme disease cases and appears within 3 days to 3 weeks following infection. The lesion may resolve spontaneously or persist for weeks to months.⁵-⁷ When present, however, the lesion is diagnostic of Lyme disease. Delaware physicians noted EM on average in 50% of Delaware cases during 1990 and 1994. This observation agrees with other published data.⁶⁻¹⁰

Delaware’s Lyme disease temporal, demographic, and numeric trends reflect regional and national data. These trends include an increasing number of reported cases, a disproportionate number of whites affected as compared with blacks, and a majority of cases occurring during the spring and summer months.

### REFERENCES

The Epidemiology of Lyme Disease and Other Tick-borne Diseases, Pennsylvania, 1990 to 1995

Perrianne Lurie, MD, MPH, FACPM

ABSTRACT

Objectives: The study was conducted to describe the temporal, geographic, and demographic characteristics of tick-borne diseases in the Commonwealth of Pennsylvania.

Methods: All physician and laboratory reports of tick-borne diseases with dates of onset from 1990 to 1995 were reviewed to determine whether they met surveillance case definitions from either the Centers for Disease Control and Prevention or the Pennsylvania Department of Health. Cases meeting the case definitions were entered into a central database for analysis. Detailed analyses of the 1993 cases were conducted.

Results: Reported cases of Lyme disease increased 53%, from 758 cases in 1990 to 1600 cases in 1995. The 1993 statewide incidence rate was 7.62 per 100,000 population. The 914 cases of Lyme disease with onset in 1993 ranged in age from 1 to 87 (mean 37.6 years, standard deviation 21.6). There was a slight preponderance of males (relative risk=1.23). The 1993 incidence rate in whites (8.32) was more than five times higher than that in blacks (1.59) and more than twice that of other races (3.30). The majority of 1993 cases had dates of onset between June and August. The incidence of other tick-borne diseases in Pennsylvania is much lower, and has remained relatively stable over time.

Conclusion: The Pennsylvania Department of Health has required reporting of several tick-borne diseases since 1987 or earlier. Lyme disease, however, probably is underreported, as Pennsylvania does not actively solicit reports of Lyme disease. The age, gender, and racial distribution of Lyme disease cases in Pennsylvania is similar to that seen in the rest of the United States and the increase in incidence and widening geographic spread of this disease parallel regional and national trends.

BACKGROUND

Tick-borne diseases, particularly Lyme disease, are a major public health problem in Pennsylvania. Lyme disease is a tick-borne, spirochetal, zoonotic disease transmitted by the deer tick Ixodes scapularis (formerly I. dammini). The spirochete Borrelia burgdorferi causes dermatologic, rheumatologic, neurologic, and cardiac signs and symptoms; most commonly, erythema migrans (EM), arthritis and arthralgia, heart block, and Bell's palsy.

The Centers for Disease Control and Prevention (CDC) initiated Lyme disease surveillance in 1982, and in January 1991, Lyme disease became reportable through the National Electronic Telecommunications System for Surveillance. Since reporting was initiated, 47 states have reported cases of Lyme disease. In 1993, Lyme disease accounted for more than 90% of all vector-borne illnesses in the United States, with 9895 cases reported. In 1994, 13,043 cases of Lyme disease were reported in the United States.

The 1993 national incidence rate was 3.8 cases per 100,000 population. Connecticut reported the highest state rate, with 61.98 cases per 100,000 population; New York State, with 5200 cases, had the highest number of cases reported in 1993.

Lyme disease has been reportable in Pennsylvania since 1987. Physicians and clinical laboratories are required to report all cases and positive laboratory tests for Lyme disease to the Department of Health.

Other tick-borne diseases are reported less commonly in Pennsylvania. All rickettsial diseases, including Rocky Mountain spotted fever (RMSF) and human ehrlichiosis are reportable. Laboratories, but not physicians, are required to report positive laboratory tests for

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Table
Number of Cases of Reportable Tick-borne Diseases in Pennsylvania, 1990 to 1995

<table>
<thead>
<tr>
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<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Lyme disease</td>
<td>758</td>
<td>958</td>
<td>991</td>
<td>1061</td>
<td>1566</td>
<td>1600</td>
</tr>
<tr>
<td>Rocky Mountain spotted fever</td>
<td>3</td>
<td>6</td>
<td>13</td>
<td>10</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Tularemia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Ehrlichiosis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

tularemia. Babesiosis, relapsing fever, and Colorado tick fever are not reportable in Pennsylvania.

The reporting system in Pennsylvania is passive and relies on voluntary compliance by physicians and laboratories. Approximately 10% of all disease reports received by the Pennsylvania Department of Health are from physicians; 90% are from clinical laboratories. No formal mechanism for patients to report cases to the Department of Health exists. The Department of Health, however, will contact the physician of any patient who attempts to report his or her own illness to determine if the patient's illness meets the case definition (see following). Every physician or laboratory report is investigated by staff at the Pennsylvania Department of Health or one of nine county/municipal health departments to determine if it meets the case definition; if it does, it is entered into the central computerized database of reportable diseases at the Department of Health.

METHODS

Surveillance data in the computerized reportable disease database from 1990 through 1995 for tick-borne diseases in the Commonwealth of Pennsylvania were reviewed and analyzed using the CDC's Epi Info software. Maps were created using CDC's Epi Map software package.

The CDC's case definition for Lyme disease is used by the Commonwealth of Pennsylvania. A confirmed case must have either EM or at least one late manifestation (recurrent brief attacks of objective joint swelling, lymphocytic meningitis, cranial neuritis, radiculoneuropathy, encephalomyelitis, or acute onset 2nd or 3rd atriioventricular conduction defects) and laboratory confirmation (by isolation of B. burgdorferi from a clinical specimen, diagnostic levels of Lyme-specific IgM and IgG in serum or cerebrospinal fluid, or significant change in Lyme-specific IgM or IgG in paired acute and convalescent sera).

CDC case definitions for RMSF and tularemia also are used by the Commonwealth of Pennsylvania. As no CDC case definition for ehrlichiosis exists, we developed a case definition for use in Pennsylvania: A suspect case of ehrlichiosis is defined as an unexplained, acute febrile illness associated with all three clinical symptoms (fever >=101°F, malaise, and headache) and at least one of the laboratory findings (thrombocytopenia, leukopenia, or elevated transaminase levels). A confirmed case of ehrlichiosis meets the definition for a suspect case and is laboratory confirmed by serology or polymerase chain reaction testing for Ehrlichia species.

Cases of reportable diseases in Pennsylvania are analyzed by date of onset rather than date of report. The data year is “closed out” on March 30 of the following year. Thus, for routine analysis, only cases with onset in a given year reported prior to April 1 of the following year are included. Cases of Lyme disease may not be reported for many months or even years after onset of the first symptom. Therefore, the routine analysis will fail to include a significant number of cases. To compensate for this time lag, updated totals for Lyme disease are prepared on an ongoing basis. The totals presented in this report reflect all cases reported through February 20, 1996.

Cases of reportable diseases with onset in 1990 to 1993 have been validated individually. Cases reported for 1994 and 1995 have not yet been validated, so some of the "cases" included in the totals for these years may not meet the case definition, thus inflating the estimates for those years. In addition, reporting for 1995 is still incomplete, leading to underestimates for that year. As the most recent year for which validated data are available is 1993, the detailed analyses were conducted for this data year.

RESULTS

A total of 6934 Lyme disease cases with dates of onset in 1990 to 1995 have been reported among residents of Pennsylvania. Fifty cases of RMSF and one case of tularemia were reported during this time period. Although we received one report of human ehrlichiosis in 1995, it did not meet our case definition. The Table shows the number of cases of reportable tick-borne diseases in Pennsylvania by year of onset.

The 914 cases of Lyme disease with onset in 1993 reported as of March 30, 1994, ranged in age from 1 to 87 (mean 37.6, SD=21.6). There was a slight preponderance of males (relative risk [RR]=1.23). Fig 1 shows the distribution of incidence rates by age and gender. The incidence rate in whites (8.32) was more than five times higher than that in blacks (1.59) and more than twice that of other races (3.30, Fig 2). The highest incidence rates in the Commonwealth were seen in the southeast, in counties surrounding Philadelphia (Fig 3). The majority of 1993 cases had dates of onset between June and August (Fig 4).

Patients with Lyme disease or their physicians were
asked detailed questions about clinical symptoms. Responses to these questions are available for only 866 of the reported cases. Of these 866, a total of 551 (63.6%) reported having EM. Fig 5 shows the distribution of these cases by month of onset. Ninety (10.4%) patients reported a history of Bell’s palsy and 364 (42.0) had a history of arthritis. Information on the prevalence of arthralgias is not available.

Patients were asked about exposure history. This information is available for only 435 of the cases. Of these, 164 (37.7%) could recall being bitten by a tick. Information on out-of-state travel was not available for analysis.

The 10 patients with RMSF with onset in 1993 ranged in age from 5 to 71 (mean 27.9, SD=24.7). There were more men than women (RR=1.5), but this difference was not statistically significant. Cases were reported from seven counties, mainly in the southeastern portion of the Commonwealth.

DISCUSSION

The number of reported cases of Lyme disease has been increasing steadily in Pennsylvania since it was made reportable in 1987. The reasons for this increase are complex and probably include increased awareness and diagnosis of the disease; ecological changes4,5 that have brought deer ticks, white-footed mice, and deer into closer contact with human beings; a population explosion among white-tailed deer in Pennsylvania6,7; and an overall increase in the proportion of infected ticks in the Commonwealth (J. Humphries, Indiana University of Pennsylvania, personal communication).

As is the case throughout the region,6-11 men outnumber women among cases of Lyme disease in Pennsylvania. The age distribution and seasonality in Pennsylvania also resemble those for the Northeast and mid-Atlantic states.

The most common presenting symptom is EM, which is not surprising as the case definition allows the inclusion of EM cases that are not laboratory confirmed. Arthritis also is a common symptom; Bell’s palsy is reported less frequently. Only 38% of cases can recall a tick bite; a proportion similar to that found in other published studies of Lyme disease.

RMSF is seen less commonly in Pennsylvania, with an average of only 10 cases a year, and no increase in the number of cases over time has been noted. As the vector of this disease is a different species of tick, Dermacentor variabilis, the fact that the incidence rates do not parallel the trends seen in Lyme disease is not surprising. The age and sex distribution is similar to that seen for Lyme disease in Pennsylvania and for RMSF nationwide.12 Behavioral risk factors leading to exposure to infected ticks, which vary by age and sex, probably account for these similarities.

As reporting of all tick-borne diseases is passive, significant underreporting of these conditions probably occurs. Studies of passive surveillance systems conducted in Vermont showed a 50% to 60% rate of underreporting for other reportable diseases when compared with active surveillance of primary care practitioners13 or hospital discharge diagnoses.14 and a recently published study in Maryland15 found that Lyme disease was underestimated by ten- to twelve-fold in 1992 and 1993. In addition, many cases of these diseases are misdiagnosed. Often, especially in the case of Lyme disease, the correct diagnosis may be delayed for months or years.

The Lyme disease surveillance case definition used by CDC and the Commonwealth of Pennsylvania undoubtedly excludes some cases of the disease. Many Lyme disease-associated conditions have been reported in the literature that are not included in the case definition, leading to an underestimation of the number of cases. Problems with the quality and interpretation of many commercial laboratory tests used in the diagnosis of Lyme disease also exist; the most frequent problem is false positive results, which would lead to overdiagnosis of Lyme disease by physicians and overestimation of the number of cases.
cases. Changes in the case definition should be considered in light of recent advances in knowledge about this disease and in the laboratory techniques used in its diagnosis. Consideration must be given to comparability of data from year to year and changes in surveillance case definitions should not be undertaken lightly.

REFERENCES

Lyme Disease Surveillance in Maryland: Sources and Outcomes of Suspected Cases

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ABSTRACT

Background: Prior to 1993, Lyme disease had been under-reported in Maryland. The Maryland Lyme disease Registry was created in an attempt to improve reporting through the use of a thorough statewide Lyme disease surveillance.

Methods: Suspected cases of Lyme disease were detected by one of three data sources including physicians, laboratories, or patients. Each case was investigated and subsequently categorized by one of five different outcomes based on data collected from the reporting physician. Cases diagnosed as Lyme disease were classified according to the Centers for Disease Control and Prevention (CDC) surveillance case definition. Cases meeting the CDC criteria were analyzed for this report.

Results: A total of 2571 suspect cases of Lyme disease were investigated during the surveillance period of April 1, 1993, through March 31, 1996. Of these, 1843 were diagnosed as Lyme disease by the reporting physicians; only 990 cases (53.7%), however, met the CDC criteria. Reporting sources for the 990 cases included laboratories (59.5%), physicians (39.2%), and patients (1.3%). Family practice, internal medicine, and pediatrics were the most common physicians' specialties reporting cases of Lyme disease. Most of the patients had month of onset (69.2%) and diagnosis (68.1%) of illness between May and August. In the majority of cases (63.1%), erythema migrans was present (mean=11.1 cm, median=10.0 cm). Joint symptoms were reported in 44.6% of the cases. A total of 1193 antibody tests were performed for 884 (89.3%) of the 990 cases.

Conclusion: Lyme disease surveillance in Maryland relies on two main reporting sources: physicians and laboratories. The incidence rate per 100,000 population increased from 3.9% in 1992 to 6.7% in 1995, suggesting that, although an actual increase in Lyme disease may exist, Lyme disease surveillance has been greatly enhanced through the coordinated efforts of the Lyme disease Registry, local health departments, the assistance of the reporting laboratories, and the participation of health care providers.

Key points: Lyme disease, state surveillance

INTRODUCTION

The first reported case of Lyme disease in Maryland was in Cecil County in 1979. Lyme disease became a reportable disease for physicians in the state in 1989. The Epidemiology and Disease Control Program of the Maryland State Department of Health and Mental Hygiene (EDCP-DHMH) adopted the Centers for Disease Control and Prevention (CDC) national surveillance case definition for Lyme disease (LLCD) in 1991. From 1979 through 1992, more than 1000 cases of Lyme disease were reported to the EDCP-DHMH. In April 1993, the EDCP-DHMH and the University of Maryland at Baltimore created the Maryland Lyme Disease Registry (LD Registry) to provide thorough statewide surveillance for Lyme disease and to supply data for an ongoing Lyme disease study on antibiotic therapy.

The EDCP-DHMH obtains reportable disease data through passive surveillance. According to an article published in 1995, the completeness of reporting in Maryland ranges anywhere from 10% for Lyme disease to 90% or more for tuberculosis. Due to the underreporting of Lyme disease cases, the registry made changes in the methods of conducting surveillance (ie, processing an increased num-
ber of laboratory reports, new Lyme disease form). Modifications to surveillance, increased awareness of Lyme disease, and higher usage of antibody testing may have precipitated changes in the sensitivity in case detection of the registry.7

This article describes the surveillance process of the Maryland LD Registry from 1993 to 1995. The source of the Lyme disease case report and the surveillance outcome for each suspected Lyme disease case received by the registry are discussed. Additionally, emphasis is placed on describing characteristics of the surveillance outcomes for Lyme disease cases that met the LDCD.

METHODS

Surveillance procedures

The registry was established in April 1993 with the collaboration of local health departments from the 24 jurisdictions in Maryland (23 counties and Baltimore City). The surveillance period covered in this article is from April 1, 1993, through March 31, 1996. March 31 was the deadline for case inclusion to assure the reporting of cases diagnosed at the end of 1995.

In April, 1993, the registry mailed a letter and new Lyme disease case report form to every physician in Maryland who had reported a Lyme disease case prior to 1993. This mailing was undertaken to introduce physicians to the Lyme disease reporting process and the form. The form was labeled with the name and address of each physician. Physicians were instructed to use copies of their blank forms to report any new Lyme disease case to the registry. Also included in the mailing were fact sheets reporting the results of analysis of 1992 Lyme disease surveillance data as an example of the type of information available through the registry and to stress the importance and benefit of reporting Lyme disease cases.

Reporting of suspected cases to the registry

Initial identification of new suspect cases of Lyme disease was through one of three data sources: physicians (DR), laboratories (LAB), or patients (PT). Physicians and laboratories mailed the initial information directly to the registry or to local health departments. Patients identified themselves through telephone calls to the registry. The data sources were categorized as follows:

Physicians (DR): Any Lyme disease cases reported by a health provider using the Maryland Confidential Morbidity Card, the Lyme disease form from the CDC, or the form from the registry were categorized as a DR report.

Laboratories (LAB): Although Lyme disease in Maryland is not a reportable disease for laboratories, many laboratories performing Lyme disease testing sent reports of patients with positive test results to the registry. These reports contained the patient’s name and the name and address of the physician who requested the test.

Patients (PT): Cases that originated from a telephone call made by a person who thought he or she, or his or her child had Lyme disease, were PT sources of data. The registry mailed a form to the physician whose name was provided by the potential Lyme disease case.

Case investigation

When a new suspect case was identified through any of the three reporting sources, the registry entered the name and address of the patient into a database. The name and address of the physician were entered into a separate database that served as a special directory. If data were incomplete, the registry called the reporting source to gather the missing information.

The registry generated a one-page individualized form with basic case information included. This form was mailed to the patient’s physician with a letter explaining the study and fact sheets reporting analysis of Lyme disease surveillance data for the preceding year. The form collected information necessary for reporting cases to the CDC and additional information needed for the study. Questions included information on tick exposure, presence of general symptoms (fever, fatigue, headache, and myalgia), size and location of the erythema migrans (EM), location of swollen joints, neurologic and cardiac symptoms, antibiotic therapy, and antibody testing for *Borrelia burgdorferi*.

If the form was not returned to the registry within 3 weeks of the date it was mailed to the physician, the physician’s office was called and prompted to complete the form and return it to the registry.

Data entry

When the form was returned to the registry, it was reviewed to ensure that all questions were answered without obvious mistakes. Physicians’ comments (ie, “not Lyme” or “not my patient”) also were reviewed. The registry then classified the case report according to one of the following outcomes:

Unable to contact: cases in which the registry was unable to get sufficient information from the physician after making several attempts at contact.

Non-Maryland cases: patients who were residents of other states but were seen by a physician in Maryland.

Non-Lyme disease cases: suspected cases in which physicians made a final diagnosis other than Lyme disease.

Old cases: any case of Lyme disease meeting the LDCD but previously reported to the EDCP-DHMH before 1993.

New Lyme disease cases: new Lyme disease cases diagnosed and reported to the registry and residing in Maryland.
Table 1
Sources and Outcomes of Suspected Cases of Lyme Disease Received by the Maryland LD Registry (1993 to 1995)

<table>
<thead>
<tr>
<th>Case source</th>
<th>Total</th>
<th>Unable to contact</th>
<th>Non-Md case</th>
<th>Non-Lyme disease case</th>
<th>Old Lyme disease case</th>
<th>New cases</th>
<th>Not met CDC</th>
<th>Met CDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physician</td>
<td>637 (100.0)</td>
<td>7 (1.1)</td>
<td>9 (1.4)</td>
<td>41 (6.4)</td>
<td>0</td>
<td>192 (30.2)</td>
<td>388 (60.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>[24.8]</td>
<td>[6.2]</td>
<td>[11.5]</td>
<td>[7.9]</td>
<td></td>
<td>[22.5]</td>
<td>[39.2]</td>
<td></td>
</tr>
<tr>
<td>Laboratory</td>
<td>1903 (100.0)</td>
<td>102 (5.4)</td>
<td>69 (3.6)</td>
<td>475 (25.0)</td>
<td>17 (0.9)</td>
<td>651 (34.2)</td>
<td>589 (30.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>[74.0]</td>
<td>[91.1]</td>
<td>[88.5]</td>
<td>[91.2]</td>
<td>[100.0]</td>
<td>[76.3]</td>
<td>[59.5]</td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>31 (100.0)</td>
<td>3 (9.7)</td>
<td>0</td>
<td>5 (16.1)</td>
<td>0</td>
<td>10 (32.3)</td>
<td>13 (41.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>[1.2]</td>
<td>[2.7]</td>
<td></td>
<td>[0.9]</td>
<td></td>
<td>[1.2][1.3]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2571 (100.0)</td>
<td>112 (4.4)</td>
<td>78 (3.0)</td>
<td>521 (20.3)</td>
<td>17 (0.7)</td>
<td>853 (33.1)</td>
<td>990 (38.5)</td>
<td></td>
</tr>
</tbody>
</table>

The registry entered a code in the patient database to record the outcome status of each case report.

Lyme disease cases meeting the LDCC

All cases categorized as "new Lyme disease cases" were entered into a separate Lyme disease database and classified according to the LDCC. Classification resulted in two groups: those meeting the LDCC (Met CDC) and those not meeting the CDC case definition (Not met CDC).

Characteristics and clinical manifestations of cases meeting the LDCC

Demographics and clinical manifestations for cases meeting the LDCC were analyzed and comparisons of the two main sources of case identification (DR and LAB) were made. Cases from PT sources were not considered because of the small size of the group.

Three major categories of clinical manifestations were created for grouping patient symptoms:

- **EM alone:** Any EM (size ≥5 cm) accompanied by associated symptoms such as myalgia, headaches, fatigue, or fever, but without Lyme disease systemic manifestation.
- **Systemic symptoms (SS) alone:** Any of the three main systemic manifestations of Lyme disease. This includes three subcategories of symptoms (symptoms in italics combined with positive antibody testing meet the LDCC. Other symptoms may be present but do not meet the LDCC): arthritic (arthritis or arthralgia); neurologic (Bell's palsy, radiculoneuropathy, lymphocytic meningitis, encephalitis, encephalomyelitis, memory problems, and optic neuritis); and cardiac (atrioventricular block, palpitations, bradycardia, bundle branch block or myocarditis).
- **EM plus SS:** Any size of EM and one or more of the SS subcategories.

Data analysis

Data analysis was performed using Epi-Info 6.0. Odds ratios (OR) and 95% confidence intervals (CI=95%) were used to compare proportions. Student’s t test was calculated for comparing means.

RESULTS

Sources

A total of 2571 suspect cases of Lyme disease seen by 1241 physicians were investigated by the registry during the period of April 1, 1993, through March 31, 1996.

Physicians reported 637 (24.8%) of the 2571 suspected Lyme disease cases (Table 1). The most common specialties of the 320 reporting physicians (DR group) were internal medicine (30.9%), pediatrics (26.3%), and family practice (23.1%). Reports from 12 different laboratories accounted for 1903 (74.0%) of the suspected Lyme disease cases. The specialties of the 902 physicians requesting laboratory antibody tests (LAB group) were internal medicine (39.0%), family practice (24.3%), and pediatrics (10.6%). Thirty one (1.2%) of the suspected cases were patients who called the registry themselves.

The three main specialties of the 19 physicians from which patient information was requested (PT group) were family practice (31.6%), internal medicine (31.6%), and pediatrics (31.6%).

Outcomes (Table 1 lists distribution of outcomes by source)

- **Unable to contact:** The registry was unable to get a response or sufficient information for 112 (4.4%) of the 2571 cases.
- **Non-Maryland cases:** Seventy-eight (3.0%) of suspected cases were residents of other states.
- **Non-LD cases:** In 521 (20.3%) of 2571 suspected cases, the physician determined the case was not Lyme disease.
- **Old cases:** Seventeen cases (0.7%) had been reported prior to 1993.
- **New LD cases:** A total of 1843 (71.7%) new Lyme disease cases was reported to the registry.
Cases meeting the LDCC

Of the 1843 new Lyme disease cases, 990 (53.7%) met the LDCC and 853 (46.3%) did not. Of the 990 cases meeting the LDCC, 388 (39.2%) were reported from DR, 589 (59.5%) from LAB, and 13 (1.3%) from PT.

Characteristics and clinical manifestations of cases meeting the LDCC

Source: Of the 637 cases from the DR group, 388 (60.9%) met the LDCC. Only 589 (30.9%) of the 1903 suspected cases from LAB met the LDCC. Thirteen (41.9%) of the 31 cases from PT met the LDCC.

Specialty of the reporting physician: The three most common physician specialties of the 990 LDCC cases were family practice (33.6%), internal medicine (33.7%), and pediatrics (17.3%). Family practitioners were 1.5 times more likely to be in the LAB group than in the DR group (CI=95%, 1.1 to 2.0, P=0.005). For physicians in internal medicine, there were no differences between the LAB and DR groups (P=0.18). Pediatricians were 3.2 times more likely to be in the DR group than the LAB group (CI=95%, 2.2 to 4.6, P=0.001).

Age, gender, and race:  The mean and median age of cases was 35 (range, 1 to 87). Cases from the DR group were younger (mean 31, median 30) than the LAB group (mean 38.2, median 38.0) (CI=95%, 5.0 to 9.9, P<0.001). Of the 990 cases, 462 (46.7%) were female and 528 (53.3%) were male. Of the 931 patients for whom race was known, 844 (90.7%) were white, 69 (7.4%) were black, and 18 (1.9%) were of other races. No differences between DR and LAB groups regarding gender and race were noted (P values 0.14 and 0.64, respectively).

Place of residence: Cases from the LAB group were 1.4 times more likely to be residents of the Eastern shore than cases from the DR group (CI=95%, 1.0 to 1.8, P=0.03). The distribution of cases by place of residence and incidence rates for each of the 24 jurisdictions in Maryland are displayed in Fig 1.

The month of onset and diagnosis: The month of onset for most of the patients (69.2%) was between May and August, with peak onset in June (27.6%). Most patients (68.1%) were diagnosed between May and August, with the peak month of diagnosis in July (24.1%). The DR group was 1.5 and 1.4 times more likely to have the month of onset (CI=95%, 1.2 to 2.1, P=0.003) and diagnosis (CI=95%, 1.0 to 1.9, P=0.02), respectively, between May and August than the LAB group.

Tick exposure: According to physician reports on the form, 407 (41.1%) of the 990 cases recalled a tick bite. There were no differences between patients recalling tick bites in the DR (41.8%) and LAB (40.6%) groups (P=0.71).

EM: In 625 (63.1%) of the 990 cases, EM was present. The mean size of the EM was 11.1 cm (median 10.0 cm, range from 2 to 52.5 cm). No statistically significant difference regarding the size of the EM between the LAB and DR groups (P>0.10) was noted. Eighty (12.8%) patients had more than one EM lesion. The most common locations of the EM were legs (34.5%), arms (20.3%), back (19.8%), and chest (13.2%). EM was 2.2 times more frequent in cases from the DR source than in those from the LAB source (CI=95%, 1.6 to 2.9, P=0.001). The proportion of pediatricians reporting EM cases was 3.0 times higher in the DR group than in the LAB group (CI=95%, 1.9 to 4.9, P=0.001). Younger age groups were more likely to be reported by the DR group than the LAB group (χ² for linear trend=14.2, P<0.001).

Arthritis and articulargia: Joint problems were reported in 442 (44.6%) of the 990 LDCC cases. A total of 260 (26.3%) had arthritis; 182 (18.4%) only had articulargia. Knees (51.3%), wrists/hands (21.0%), and elbows (11.6%) were the most afflicted joints. Cases from LAB reports had 1.6 times more arthritis that those from the DR group.

Neurologic manifestations: Patients with neurologic problems were 1.8 times more likely to be in the LAB group than in the DR group (CI=95%, 1.3 to 2.5, P<0.001).

Cardiac manifestations: Cardiac symptoms such as atrioventricular block, palpitations, and bradycardia were reported more commonly in the LAB than DR group (P=0.18).

Associated symptoms: No statistically significant difference between the LAB and the DR groups regarding general symptoms such as headache, myalgia, fever, or fatigue (P=0.20) were noted.

Categories of clinical manifestations: The EM-only category was 1.8 times more frequently reported by the DR group than the LAB group (CI=95%, 1.4 to 2.4, P<0.001). The EM plus SS category was evenly reported by the DR and the LAB groups (CI=95%, 0.9 to 1.7, P=0.21). The SS-only category was 2.2 times more likely to be reported from LAB than from DR (CI=95%, 1.7 to 2.9, P<0.001). Fig 2 (Venn diagram) displays the clinical findings for the 990 cases meeting the LDCC. Table 2 contains clinical manifestations by source of suspected cases.
**Antibody testing:** Of the 990 cases meeting the LDCC, 884 (89.3%) had 1193 tests at the time of diagnosis (1.4 tests per case). Of the 1193 tests performed, 86.9% were screening tests and 13.1% were confirmatory tests. A total of 86.9% of the 1193 tests were positive, 11.6% were negative, and 1.5% were equivocal. Of the specimens sent to the laboratory, 96.0% were serum, 3.5% were cerebrospinal fluid, and 0.5% were another type.

All 589 cases meeting the LDCC from the LAB group had antibody testing, with 216 (36.7%) having two or more tests at diagnosis (1.4 tests per case). In the DR group, only 287 (74.0%) of the LDCC cases had antibody testing prior to diagnosis and reporting. Results for the first test were negative 3.5 times more frequently in the DR group than in the LAB group (CI=95%, 2.2 to 5.5, P<0.001).

**DISCUSSION**

These findings show that Lyme disease surveillance in Maryland relies on two main reporting sources: physicians and laboratories. Although the DR source represents only 24.8% of all Lyme disease suspected cases, 60.9% of these cases met the LDCC. The LAB source represents 74.0% of the 2571 suspected cases, but only 30.9% met the LDCC. An explanation for this result is the number of other illnesses that present a false-positive reaction to the screening test, as supported by the 475 (25.0%) non-Lyme disease cases out of the 1903 suspected cases from the LAB group.

Possible cases that the registry was unable to contact (4.3%) are considered a normal lack of response or lost to follow up. Despite cases that could not be contacted, the registry had a response rate (95.7%) much higher than previous Lyme disease surveillance in Maryland (87.8% in 1991 and 87.5 in 1992). The majority (91.1%) of cases unable to be contacted were from the LAB group, which often meant that the patient did not return to the physician’s office for final diagnosis. In other situations, the physician was hesitant to diagnose and report a case of Lyme disease.

The registry was unable to determine cases that were from other states until the patient address was submitted on the form. Most of these patients sought medical care in Maryland and had submitted a serum sample to a laboratory while under the care of a Maryland physician. The laboratories reported these cases to the registry, assuming the patient was a resident of Maryland.

The high proportion of non-Lyme disease cases reported by LAB (91.2% of total non-Lyme disease cases) indicates that many physicians use antibody testing to rule out other health problems. More than 25% of the time such a test was requested, specialties and subspecialties such as neurology, orthopedic surgery, rheumatology, gynecology, and cardiology concluded that the patient did not have Lyme disease. Another explanation is the availability of confirmatory tests (i.e., Western blot). A negative confirmatory test after a positive screening result (reported to the registry by the laboratory) might change the initial diagnosis of Lyme disease.

All LD cases determined as old came from the LAB source due to a medical follow up in which a lab test was requested. The registry was able to determine cases diagnosed and reported before 1993 on return of the form. The form included dates of onset and diagnosis, which were not included on the laboratory report. The registry compared these dates against previously reported cases.

Although only 30.9% of LAB cases reported met the LDCC, this source represents the backbone of Lyme disease surveillance in Maryland, contributing 59.5% of the total cases meeting the LDCC. Due to underreporting of Lyme disease cases in Maryland, the registry began thorough case investigations on all laboratory reports. Because of this approach, the incidence rate of Lyme disease per 100,000 population in Maryland increased steadily from 3.9 in 1992 to 4.2 in 1993 to 6.9 in 1994 and to 6.7 in 1995 (registry records). This increment may reflect not only increased surveillance but an actual increase in Lyme disease cases. This pattern observed in Maryland is similar to the national trend.

Of the 990 cases meeting the LDCC, 827 (83.5%) were reported by primary care physicians (internal medicine, family practice, pediatrics, emergency room) and 122 (12.3%) by physicians from other specialties and subspecialties such as orthopedic surgery, rheumatology, neurology, dermatology, and cardiology. Similar results were found previously in Maryland and Connecticut. The high number of primary care physicians reporting Lyme disease indicates that even though primary care physicians often refer patients to specialists (i.e., a neurologist, rheumatologist, surgeon, or cardiologist) for a second opinion, the primary care physician makes the final diag-
nosis and reports the case.

Patients in the DR group were younger than in the LAB group, which correlates with the higher distribution of pediatricians in this group. The distribution by gender, race, recollection of tick bite, and seasonal onset of Lyme disease was similar to the distribution found in 1992.5

The high proportion of reported cases diagnosed during the warmer months suggests that physicians from the DR group saw more acute cases (EM) during this period than physicians from the LAB group. In contrast, most of the cases from the LAB group had onset late in the summer or thereafter; the physician awaited the results of antibody testing. The higher number of cases reported from the LAB group presenting systemic manifestations (arthralgia, neurologic, and cardiac problems) without the presence of EM indicates that the lab report was received by the case investigation ensued before the physician made a final diagnosis.

Based on the results of this analysis, Lyme disease surveillance in Maryland has been greatly enhanced through the coordinated efforts of the registry, local health departments, the assistance of the reporting laboratories, and the participation of health care providers. The registry has increased awareness of Lyme disease through dissemination of information, which has led to an increased reporting by physicians throughout Maryland and surrounding states.

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REFERENCES
Surveillance of Tick-borne Disease in Selected States

Julie A. Rawlings, MPH; John Pape; William Bigler, PhD; Jeff Berschling; Jesse F. Greenblatt, MD, MPH; Joan Bestudik; Gianfranco Pezzino, MD, MPH; Jim Murphy; Larry A. Shireley, MS, MPH; Craig R. Nichols, MPA; Suzanne R. Jenkins, VMSD, MPH; Marcia Goldoff, MD; and James J. Kazmierczak, DVM, MS

In anticipation of publishing an issue of the Journal of Spirochetal and Tick-borne Diseases focusing on surveillance, a letter requesting tick-borne disease data was sent to each of the 50 states. A manuscript containing detailed epidemiologic information was solicited from the states considered “endemic” for Lyme disease. Epidemiologists in “nonendemic” states were asked to answer several questions regarding their experiences with tick-borne diseases.

The Journal received manuscripts from Delaware, Maryland, Massachusetts, and Pennsylvania. Tick-borne disease surveillance information was submitted from an additional 13 states: Colorado, Florida, Georgia, Idaho, Illinois, Kansas, Montana, North Dakota, Texas, Utah, Virginia, Washington, and Wisconsin. Although only 17 states elected to participate, most areas of the United States are represented.

Lyme disease and Rocky Mountain spotted fever (RMSF) are notifiable conditions in all 13 of the states who responded; tularemia is reportable in 11; tick-borne relapsing fever (TBRF) is reportable in four; Colorado tick fever is reportable in three; ehrlichiosis is reportable in two; and tick paralysis is reportable in one. Although not reportable, Washington identified its first case of babesiosis in 1991.

Lyme disease was made reportable as early as 1984 (Wisconsin) and as late as 1991 (Florida). Other tick-borne diseases have been reportable for longer periods of time. For example, RMSF or tularemia were reported in Florida, Utah, and Washington during the 1920s. Tularemia was reported in Texas during the 1920s and RMSF and TBRF were reported in the 1930s.

Reporting requirements differ from state to state. For instance, in Colorado, Utah, and Washington, five tick-borne disease are reportable (no tularemia cases in Utah have been associated with tick bites; tularemia has been associated only with deerfly bites and handling infected rabbits). Four tick-borne diseases are reportable in Florida, Idaho, Montana, and Texas; three are reportable in Georgia, Illinois, North Dakota, Virginia, and Wisconsin. In many states, penalties can be imposed for not reporting, although these provisions are rarely, if ever, used. In Colorado, reporting of ehrlichiosis and tick paralysis is encouraged but not mandated, and cases are documented. When received, ehrlichiosis cases are recorded in Virginia; ehrlichiosis soon will become reportable in Wisconsin.

Montana and Virginia use a combination of active and passive tick-borne disease surveillance. Surveillance for tick-borne diseases in Colorado, Florida, Illinois, North Dakota, Texas, Utah, Washington, and Wisconsin is entirely passive. Nearly 100% of Lyme disease reports come from physicians in Utah and Wisconsin. In Washington, Lyme disease, TBRF, and tularemia are reportable diseases for health care practitioners. Only tularemia is reportable for laboratories. In Texas, although physicians are required to report, tick-borne disease surveillance is primarily laboratory-based. In North Dakota, laboratory reporting was not mandatory until July 1, 1996.

Each of the states uses the Centers for Disease Control and Prevention (CDC) case definition to confirm Lyme diseases cases. Minor variations exist. For instance, before a case can be credited to Colorado, it must meet the CDC criteria and have been acquired in the state. Imported cases may be referred to the state of acquisition but are not counted as Colorado morbidity for national statistical purposes. On the other hand, states such as Texas will count

From the Texas Department of Health (Ms Rawlings); the Colorado Department of Public Health and Environment (Mr Pape); the Florida Department of Health and Rehabilitative Services (Dr Bigler); the Georgia Department of Human Resources, Division of Public Health (Mr Berschling); the Idaho Department of Health and Welfare (Dr Greenblatt); the Illinois Department of Public Health (Ms Bestudik); the Kansas Department of Health and Environment (Dr Pezzino); the Montana Department of Public Health and Human Services (Mr Murphy); the North Dakota State Department of Health and Consolidated Laboratories (Mr Shireley); the Utah Department of Health (Mr Nichols); the Virginia Department of Health (Dr Jenkins); the Washington Department of Health (Dr Goldoff); and the Wisconsin Department of Health and Social Service (Dr Kazmierczak).

Address reprint requests to Julie A. Rawlings, MPH, Texas Department of Health, Infectious Disease Epidemiology and Surveillance (IDEAS), 1100 West 49th St, Austin, TX 78756-3194.
<table>
<thead>
<tr>
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<td>Tularemia</td>
<td>15</td>
<td>7 to 76</td>
<td>9 M, 6 F</td>
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</table>

*A total of 1229 possible cases were reported.

M=male; F=female; RMSF=Rocky Mountain spotted fever; TBRF=tick-borne relapsing fever; N/A=not available.

Fig: Reported cases of Lyme disease by county of residence, Wisconsin, 1991 to 1995 (n=2128).

all cases in residents no matter where the infection was acquired. Several respondents proposed that the CDC case definition be improved. Each of them suggested strengthening the criteria for laboratory confirmation. In addition, one respondent recommended distinguishing between imported and indigenous cases to reflect the distribution of Lyme disease more accurately.

Conceding that few, if any, surveillance systems are completely satisfactory, the authors are generally happy with their state's system. Several thought that mandatory laboratory reporting would increase the number of case reports and allow for more timely case investigations. On the other hand, they acknowledged that laboratory reporting will not be practical until more standardized, accurate assays are available.

The demographic characteristics of tick-borne diseases for selected states are contained in the Table. The number of reported cases of Lyme disease by county of residence in Wisconsin is shown in the Figure. This information is included to remind medical professionals that many tick-borne diseases continue to occur in the United States.

As international travel becomes increasingly commonplace, along with an expanded risk for importing and exporting disease, health care practitioners should remember that the diseases discussed here occur in other parts of the world. Perhaps a future issue of the Journal of Spirochetal and Tick-borne Diseases will focus on surveillance for tick-borne infections outside the United States.
Clinical Conference: Chronic Lyme Disease
Sam T. Donta, MD; Elizabeth Aberer, MD; and Martina H. Ziska, MD

INTRODUCTION

The presentations at the Ninth Annual International Conference on Lyme Borreliosis and Other Tick-borne Disorders, Boston, Mass, April 19-20, 1996, focused on "Chronic Lyme Disease: Basic Science and Clinical Approaches." The term "chronic Lyme disease" is being used to describe the long-term, frequently ongoing, symptoms and sequelae of infection with Borrelia burgdorferi. Patients with chronic Lyme disease may manifest objective findings (eg, arthritis), but much more commonly have subjective symptoms of encephalopathy, peripheral and radicular neuropathy, arthralgias or myalgias, and fatigue.

ANIMAL MODELS OF CHRONIC LYME DISEASE

In this session, mouse, hamster, and rhesus monkey models were reviewed. In both the mouse and hamster models, the joint manifestations are prominent; in contrast, the neurologic system seems to be the primary target of infection in the monkey. Stephen Barthold (Yale University, New Haven, Conn) noted that the OspA protein of infecting spirochetes is downregulated when ticks begin to take a blood meal, and that other proteins are expressed that probably are required for tissue invasion in the mouse. Host humoral, but not cellular, immune responses can protect against and help resolve infection initiated by intraperitoneal inoculation, but immune serum does not protect against natural, tick-mediated infection. Differences in the susceptibility of inbred strains of mice should provide greater insight into host defenses against B. burgdorferi infection.

Ronald Schell (University of Wisconsin, Madison) described the chronic synovitis that occurs in the hamster following intraarticular injection. A severe arthritis occurs when hamsters previously vaccinated with a killed whole cell preparation of B. burgdorferi are challenged with any of six different serogroups of B. burgdorferi. CD4 cells appear to be responsible for the development of arthritis, and CD8 cells may be involved in the milder form of synovitis that occurs in nonvaccinated infected hamsters. The results of this work could have implications for the development of safe vaccines.

Mario Philipp (Tulane University, New Orleans, La) reviewed the development of humoral and cellular immune responses and the various organs involved in chronic Lyme disease in the monkey. By Western blot analysis, antibodies against the 39kd protein of B. burgdorferi were particularly prominent by 10 weeks postinfection. In the monkey, an erythema migrans (EM) rash develops at the site of inoculation, characterized by perivascular and perineural infiltrates. A chronic arthritis, with synovial hyperplasia and periarticular fibrosis similar to that seen in the human disease, was noted. The neurologic involvement included necrosis of sensory ganglia, a peripheral neuropathy characterized by perivascular infiltrates, and a chronic meningitis. Live spirochetes were recovered in the first 10 weeks, but none could be cultured in the chronic stages.

PATHOGENESIS OF CHRONIC LYME DISEASE

In this session, five presentations focused on mechanisms that may be involved in the persistence of B. burgdorferi in vivo. Alan Barbour (University of Texas, San Antonio) reviewed several possibilities and findings that would explain or support the persistence of the organism: poor immunogenicity, antigenic variation, intracellular localization, niches (brain, eye), and the presence of microbial DNA. Other borrelial species are known to undergo antigenic variation to avoid the host immune response (eg, Borrelia hermsii) or to occupy special niches (eg, Borrelia turcoi). Analogies of some of B. hermsii polymorphic proteins to the OspC proteins of B. burgdorferi exist.

From the Boston University Medical Center (Dr Donta); the University of Graz Austria (Dr Aberer); and Lyme Disease Foundation (Dr Ziska), Hartford, Conn.

Address correspondence to Sam T. Donta, MD, Boston University Medical Center, 88 E. Newton St-Evans, 6th Floor, Boston, MA 02118.
Janet Weis (University of Utah, Salt Lake City) presented evidence of less spirochetal DNA in tissues of infected BALB/c mice compared with C3H/HeJ mice to help explain the differences seen in clinical disease between these two inbred species. Nitric oxide, which is highly toxic to *B. burgdorferi*, was increased in the urine of both species of mice 7 to 10 days postinfection; inhibition of nitric oxide formation, however, did not alter the kinetics and quantity of spirochetes found in tissues or the clinical disease. These results suggest that this cytokine might not play a primary role in the control of the disease. In studies of pregnancy, mice with chronic infection had no abnormal fetal outcomes compared with those infected during pregnancy (12% fetal deaths).

David Dorward (National Institutes of Health [NIH], Rocky Mountain Laboratories, Hamilton, Mont) detailed the interactions of *B. burgdorferi* and human B and T lymphocytes in vitro. The spirochetes were shown to attach to, invade, and kill lymphocytes. Intracellularly, they were observed in vacuoles that did not fuse with lysosomes. Spirochetes emerging from lymphocytes were surrounded by host cell membrane, suggesting a mechanism by which the bacteria could avoid recognition by the immune system.

Mark Klemperer (Tufts University, Boston, Mass) demonstrated that *B. burgdorferi* binds to host proteases (eg, plasminogen activator), using these to help invade tissues and to perhaps evade immune responses.

Elizabeth Aberer (University of Graz, Austria) examined the reasons for borrelial persistence in the chronic skin lesions of Lyme disease, ie, acrodermatitis chronica atrophicans, and found that the Langerhans cell, the most important cell for local antigen presentation, is heavily damaged. MHC class II molecules were strongly downregulated on Langerhans cells in both early and late Lyme disease. These findings might help to explain the impaired capacity of the host to eliminate *B. burgdorferi* from skin sites.

**LABORATORY CONFIRMATION OF CHRONIC LYME DISEASE**

The laboratory diagnosis of Lyme disease has suffered primarily from a lack of sensitivity of existing tests; the diagnosis relies on clinical criteria. The role of humoral immunity in preventing the establishment of chronic disease also is in question. In this session, the various serologic tests and PCR assays were examined for sensitivity and specificity. In addition, the antiborrelial activity of serum from patients with late Lyme disease was reported by Charles Pavia (New York Medical College, Valhalla) to protect mice from infection; sera from patients with early Lyme disease failed to provide protection. All of the late disease sera had borrelial activity; in contrast, 30% to 40% of early disease sera had any borrelial activity. Pavia also detailed the sequential human immune responses to infection, noting the absence of antibodies to the OspA (31kd) and OspB (34kd) proteins during the EM stage, and an expansion of antiborrelial responses in untreated patients with EM that correlated with the duration of the rash.

Richard Tilton (BBI-North American Laboratory Groups, New Britain, Conn) reported that the use of the Centers for Disease Control and Prevention (CDC) criteria for standardization of Lyme disease Western blot serologies results in the underdiagnosis of patients with Lyme disease because: a) the CDC criteria rely on greater numbers of reactions than are seen in many patients; and b) the CDC criteria fail to include certain responses (eg, 31kd, 34kd, 83kd) that are highly specific reactions. Tilton noted that some test strains do not express all of the antigens. Based on the available information, it was concluded that the criteria should be reexamined and revised to focus on specific reactivities rather than numbers of reactions.

Both Mark Manak (Biotech Research Laboratories, Bethesda, Md) and Bruno Schmidt (Ludwig Boltzmann Institute, Vienna) emphasized the value of PCR-DNA analyses in the diagnosis of Lyme disease. In prospective studies, Manak reported that 11 of 22 untreated patients with Lyme disease had OspA-specific gene sequences detected in plasma and buffy coat preparations; within 1 week of treatment, most of the patients became PCR negative. The buffy coat preparations appeared to be more sensitive than plasma. No good correlation between PCR assays and seroreactivity was noted. Overall, patients with chronic Lyme disease have a lower incidence of PCR positivity. Schmidt reported that nested PCR assays gave greater reactivities, especially in urine specimens. He noted that 89% of patients with EM were urine PCR positive; this positivity decreased to 20% in patients with chronic Lyme disease.

**PREVENTION OF LYME DISEASE**

This session focused on the two Lyme disease vaccines in human clinical trials. Francois Meurice (SmithKline Beecham, Philadelphia, Pa) and John Zahradnik (Connaught Labs, Swiftwater, Pa) both reported on their Phase I and Phase II trials of a recombinant OspA vaccine. Meurice noted that the vaccines appeared to be safe, including patients with a prior history of Lyme disease. Reactions including headache, fatigue, and arthralgias were reported by 40% or less of these patients. Phase III trials have been initiated, using two injections of 30 µg of OspA protein. Zahradnik noted that his company’s vaccine protected dogs against tick infection, and that phase 1 and phase 2 studies demonstrated the safety of the vaccine. In dose-escalation studies, 30 µg provided the greatest responses. Headaches, fatigue, and arthralgias were noted in patients with or without known prior Lyme disease; this incidence was no higher in those receiving
the vaccine when compared with those receiving placebo. A phase 3 study is in progress that uses 10 200 subjects and an immunization schedule of two monthly injections followed by a booster 1 year later.

EMERGING TICK-BORNE DISEASES

In recent years, it has been appreciated that other potential human pathogens can be transmitted by the bite of the Ixodes tick. Louis Magnerelli (Conn Agricultural Station, New Haven) reported that 50% of adult Ixodes scapularis ticks sampled from Connecticut contained DNA sequences of the human granulocytic ehrlichiosis (HGE) agent. The rickettsial agent of HGE, ie, Ehrlichia equi, and the agent of HME (human monocytic ehrlichiosis), ie, Ehrlichia chaffeensis, can be found in other tick species (eg, Dermacentor, Amblyomma) as well. In persons with Lyme borreliosis, antibodies to either ehrlichial species or Babesia microti, the parasitic agent of babesiosis, were detected in 8% to 20% of their sera. Magnerelli speculated that more organisms remain to be discovered.

David Persing (Mayo Clinic, Rochester, Minn) reported that 9% to 20% of patients from the northeastern United States with Lyme disease have been coinfected with Babesia. He speculated whether the parasitic infection might lead to some immunosuppression, with a decreased host immune response to the Lyme spirochetes, an increased frequency of PCR positivity for spirochetal DNA, and a more severe Lyme disease. This hypothesis has not yet been validated. He also reported evidence that HGE has been present since the 1970s in the Midwest.

Stephen Dumler (University of Maryland, Baltimore) detailed the clinicopathologic features of HGE and HME, and compared them with Lyme disease. The Ehrlichia are capable of intracellular persistence, and phagolysosomal fusion is blocked by these rickettsiae. They have been known to cause diseases in horses, goats, and dogs, and were only recently discovered to be the cause of a severe, acute illness in humans. This illness is characterized by fever, headaches, myalgias, leukopenia, and thrombocytopenia. The diagnosis is made on clinical grounds, as serologic responses may not be positive in the acute phase. In patients with Lyme disease, an average of 13% have antibodies to HGE. The roles of coinfection and persistence of HGE and HME must be evaluated.

CLINICAL DIAGNOSIS OF CHRONIC LYME DISEASE

Nancy Shadick (Harvard University, Cambridge, Mass) reported the results of a long-term follow up of patients with Lyme disease in Nantucket. She found a higher prevalence of arthralgias, fatigue, and memory/word finding difficulties in patients compared with controls. Almost one third of patients reported relapses after initial treatment.

Martina Ziska (Lyme Disease Foundation, Hartford, Conn) found that none of nine women who had chronic Lyme disease and then became pregnant suffered any adverse fetal outcomes. All of these women, however, were treated during their pregnancy. The prevailing thinking is that the risk to the fetus occurs if the woman is infected during the early part of her pregnancy. Further studies are needed to better define the incidence of adverse outcomes.

Kenneth Liegner (private practice, Armonk, NY) used four of his cases to demonstrate the clinical spectrum of the chronic meningoencephalomyelitis. All four cases were initially seronegative, but positive by culture or other CSF antigen or antibody detection. They all had deteriorating neurologic function: one patient with hydrocephalus died, another satisfied the diagnostic criteria for multiple sclerosis, and two others with relapsing clinical courses eventually became seropositive and responded to prolonged antibiotic therapy. The frequency of these types of cases is unknown. Liegner concluded that some current tenets likely are incorrect, ie, that short-term antibiotic therapy is effective in the treatment of chronic Lyme disease; the differentiation between multiple sclerosis and Lyme disease is simple; patients with Lyme disease always have positive serologic tests; and CSF antibody analysis is a reliable means of diagnosing CNS Lyme disease.

Patricia Coyle (State University of New York [SUNY], Stony Brook) detailed the various late Lyme disease neurologic syndromes. The most common is an encephalopathy; the others include a chronic polyradiculoneuropathy (eg, paresthesias) and an encephalomyelitis. Symptoms prevail and clinical signs are uncommon. Patients may be seropositive or seronegative, although Coyle believes that detailed CSF analyses, some using experimental procedures (eg, OspA antigen detection), may be helpful.

Brian Fallon (Columbia University, New York, NY) reviewed the neuropsychiatric manifestations of Lyme disease, noting that depressive and cognitive disorders predominate and can become difficult to diagnose as Lyme disease. In controlled studies, these manifestations were three times as likely to occur in Lyme disease compared with patients with systemic lupus or other arthritides. Simple cognitive tests (eg, Buschke selective reminding) can be helpful in documenting dysfunction. SPECT scans are proving helpful in the diagnosis of CNS Lyme disease; 52% of 35 patients studied with Lyme disease had abnormal blood flow patterns, primarily to the frontal and temporal cortical regions. The specificity of these patterns is uncertain, although typical in chronic Lyme disease. The pathophysiologic basis for these changes remains to be defined.

Leslie Fein (private practice, West Caldwell, NJ)
reported on her clinical experience with chronic Lyme disease patients. She noted that many patients were misdiagnosed with autoimmune disorders, fibromyalgia, chronic fatigue syndrome, and Epstein-Barr virus disease. Patients with Lyme disease frequently had reactivities to cardiolipin, rheumatoid factor, and nuclear antigens, which reactivities disappeared or diminished with treatment. Seropositivity in patients increased from 70% at initial diagnosis to 89% with long-term treatment. Improvement of clinical symptoms correlated with duration of antibiotic treatment: 15% improved with up to 2 months of therapy, 32% with 2 to 3 months of therapy, and 54% with 6 months of therapy. Repeat courses of therapy also increased the incidence of improvement.

TREATMENT OF CHRONIC LYME DISEASE

Benjamin Luft (SUNY, Stony Brook, NY) reviewed the evolution of understanding of chronic Lyme disease. Key factors include heterogeneity of the spirochetal agent, the possible association of different genospecies with differing clinical manifestations (eg, neurologic vs rheumatic), and differing host responses and expression of clinical disease. He also cited the difficulties in making the diagnosis because of generally nonspecific signs and the need for better serologic and other assays, necessitating making the diagnosis on primarily clinical grounds.

Claude Garon (NIH, Rocky Mountain Laboratories, Hamilton, Mont) described the effects of melittin, a small polypeptide found in bee venom, on Borrelia burgdorferi. On exposure to micromolar concentrations, the spirochetes became immotile and surface membranes became unglued, with bleb formation, followed by death of the organism. Further research will be needed to examine any potential role of melittin in the control of Lyme disease.

Sam Donta (Boston University, Mass) reported on his results of treating 277 patients with clinically defined chronic Lyme disease with tetracycline. Patients who were seronegative had similar rates of cure, improvement, or failure as seropositive patients. Of note, enzyme immunoassays (EIA, ELISA) were much less sensitive (negative in >50% of patients with positive Western blots) than were Western blot assays, supporting the earlier presentations in the conference that the currently recommended CDC criteria, ie, performing Western blots only to confirm positive ELISA tests, are inappropriate and result in an underdiagnosis of Lyme disease. A key finding was the correlation between the prior duration of symptoms and treatment outcome, ie, patients with longer durations of symptoms prior to treatment had slower response times (ie, onset of improvement), lower cure rates, and higher failure rates. Overall, most patients (90%) were cured (no recurrences of any symptoms for >1 year) or improved with a course of tetracycline treatment (500 mg tid) that averaged 4 months (range 1 to 11 months). A second course of treatment with tetracycline led to further cures or improvement in most patients. Controlled trials will be needed to validate these observations.

Louis Corsaro (Columbia University, New York, NY) reported on his experience with intramuscular benzathine penicillin in the treatment of 25 children with chronic Lyme disease, most of whom were seropositive. Most of these patients had been treated previously with IV or oral antibiotics, usually beta-lactam antibiotics. The duration of IM treatment ranged from 4 to 38 weeks, and resulted in improvement in all patients.

Joseph Burrascano (private practice, East Hampton, NY) detailed his experience with vancomycin in 19 patients who had failed other therapies. Vancomycin was administered twice weekly for an average of 7 weeks. Two failures and three cures were achieved; the remaining patients had good or partial responses. Burrascano concluded that vancomycin was a "salvage" protocol to be used when other agents have failed.

NIH INTRAMURAL-EXTRAMURAL COLLABORATIVE STUDY OF CHRONIC NEUROBorreliosis

The final presentation of the conference was by Adriana Marques. She reviewed an upcoming study of the NIH that would examine seropositive patients who have neurological symptoms for more than 3 months, including routine and specialized laboratory tests, in the hope of better understanding the disease. Patients would also be offered treatment with 4 weeks of IV ceftriaxone and followed for an additional year after therapy. In the discussion section, criticism of the proposed 4-week treatment duration was expressed, as evidence presented at the conference supported longer duration treatment periods. The
INFORMATION FOR CONTRIBUTORS

Journal of Spirochetal and Tick-borne Diseases

Dedicated to science and art in spirochetal and tick-borne diseases

INFORMATION FOR AUTHORS AND EDITORIAL POLICY

The following guidelines are in accordance with the “Uniform Requirements for Manuscripts Submitted to Biomedical Journals” and the International Committee of Medical Journal Editors (the “Vancouver Group”) statement, agreed at the January 1993 Meeting.

The *Journal of Spirochetal and Tick-borne Diseases* publishes quarterly reviews and original work studies about any aspect of spirochetal and tick-borne diseases. The primary purpose is to broaden our understanding of spirochetal and tick-borne diseases. Special focus is given to Lyme borreliosis (also known as Lyme disease), as the most prevalent spirochetal and tick-borne disease. The clinical topics may involve all medical disciplines, nursing, and pharmacy, as well as the social, ethical, and biological features of spirochetal and tick-borne diseases.

The Journal is composed of two major sections. One section is devoted to the review of a specific topic that is established by the Associate Editors, and a special guest editor is invited to coordinate the development of up to six manuscripts relating to the specific topic. The second section of original works is composed of unsolicited manuscripts that are subsequently reviewed by the Review Board, as well as external reviewers, depending on the potential for conflict of interest within the editorial panel and the potential interest by the readership.

Expedient review of all manuscripts is carried out with a projected response time of not more than 6 weeks. Rejected manuscripts usually are returned to authors within 6 weeks. Decisions about potentially acceptable manuscripts may take somewhat longer.

The Journal will publish material defined within the categories described in the following.

Reviews

Each issue includes a series of state-of-the-art articles on a topic related to spirochetal and tick-borne diseases. The articles represent invited presentation by authorities in the field on topics related to spirochetal and tick-borne diseases, with an emphasis on Lyme borreliosis.

Submissions to this category should present a comprehensive state-of-the-art analysis and should be accompanied by an abstract of 300 words or less summarizing major points.

Peer Review Articles

Original articles of 5000 words or less may be submitted to the editorial office. Each article should be accompanied by an abstract of 300 words or less describing the findings of the original research. All articles will be peer reviewed within a 3-week period with subsequent notification to the authors within 5 weeks of submission.

Case Reports

Specific clinical case reports describing a unique approach to Lyme disease and other related disorders in the area of diagnosis or treatment may be submitted for review. An abstract of 250 words or less should accompany the text.

Correspondence

Letters to the Editor in the form of correspondence related to material published in the Journal or some aspect of Lyme borreliosis and other spirochetal and tick-borne diseases may be submitted. Such letters, if related to work previously published in the Journal, will be referred to the author of the original work for a response. Letters to the Editor should be submitted in duplicate, typewritten and double-spaced, not exceeding 400 words of text and a maximum of five references. Letters should have no more than three authors, and should be signed by all of the authors. Please include a word count.

Receipt of letters is not acknowledged, but correspondents will be notified when a decision regarding publication is made.

Editorials

Editorials may be published, usually at the solicitation of the Associate Editors, but unsolicited submissions that relate to an unusual topic of interest exceeding the usual designation of correspondence, i.e., 1000 words or less, will be considered.
Photographic Section

The topical photographic section will be a regular feature. Photographs pertinent to articles presented in the Journal, as well as other photographs related to any aspect of spirochetal or tick-borne diseases, will be considered for the publication. The guidelines for the submission are designated in Illustrations.

Conflict of Interest

The Journal asks authors to disclose at the time of submission any financial or other arrangements they may have with a company whose product figures in the submitted manuscript or with a company making a competing product. Such information will be held in confidence while the paper is under review and will not influence the editorial decision, but if the article is accepted for publication, the editors will discuss with the authors the manner in which such information is to be communicated to the reader.

Because the essence of reviews and editorials is selection and interpretation of the literature, the Journal expects that authors of such articles will not have any financial or other interest in a company (or its competitor) that makes a product discussed in the article. Potential authors who have questions about these issues should contact the Managing Editor.

Submission of Manuscript

An original and three copies of the manuscript should be submitted to:

Karen Forschner
Business Manager
Journal of Spirochetal and Tick-borne Diseases
Lyme Disease Foundation
1 Financial Plaza
Hartford, CT 06103
Telephone: (806) 525-2000
FAX: (806) 525-8425
e-mail: LymeFnd@aol.com

Manuscripts containing original material are accepted with the understanding that neither the article nor any part of its essential substance has been or will be published or submitted for publication elsewhere before appearing in the Journal.

All manuscripts should be accompanied by a letter of copyright transmittal. This must be signed and dated by all authors. The letter is required before any manuscript can be considered for publication and should contain the following wording:

“In consideration of The Lyme Disease Foundation taking action in editing my (our) submission, the author(s) undersigned hereby transfers, assigns, or otherwise conveys all copyright ownership to The Lyme Disease Foundation. The copyright so conveyed includes any and all subsidiary forms of publication, such as electronic media. The author(s) declares that the manuscript contains no matter that is, to the best of the author’s knowledge, libelous or unlawful, or that infringes upon any U.S. copyright.”

All manuscripts should be submitted with a cover letter indicating the category for which the manuscript should be reviewed. Copies of any closely related manuscripts should be submitted to the Editor along with the manuscript that is to be considered by the journal.

A cover letter, signed by all authors, should identify the person (with the address and telephone number) responsible for negotiations concerning the manuscripts; the letter should make it clear that the final manuscript has been seen and approved by all authors and that they have taken due care to ensure the integrity of the work. Manuscripts should include a title page, abstract, and text, with tables, illustrations, and references below.

For the integrity of the published material, manuscripts describing clinical aspects of Lyme borreliosis must disclose criteria for patient enrollment into the study and criteria for defining “successful” or “nonsuccessful” Lyme borreliosis treatment.

Manuscripts without these requirements will be automatically rejected.

Titles and Author’s Names

With the manuscript, provide a page giving the title of the article; titles should be concise and descriptive (not declarative). Also include a running head of fewer than 40 letter spaces; the name(s) of the author(s), including the first name(s) and academic degree(s); the name of the department and institution in which the work was done; the institutional affiliation of each author; and the name and address of the author to whom reprint requests should be addressed.

Any grant support that requires acknowledgment should be mentioned on this page.

Abstract

Provide on a separate page an abstract of not more than 300 words (original and review articles) or 250 words (case report). This abstract should consist of four paragraphs, labeled Background, Methods, Results, and Conclusion. They should briefly describe the problem being addressed in the study, how the study was performed, the results, and what the authors conclude from the results.

Text

All material should be typed and double-spaced. Standard sequence of methods and materials, results, and discussion should be employed with tables and figures numbered in the order in which they are cited in the text. A disk in text format should accompany this.

Tables

Submit tables typed and double-spaced and provide a heading for all columns with a comprehensive title on separate sheets. A disk copy with a
separate file for each table should be on the disk containing the text.

Illustrations
Photographs and figures should be submitted as glossy prints 5×7 in., with one copy of each print for each copy of the manuscript. Figure legends should be provided on a separate sheet with identification of the figure. The back of the glossy print should indicate the number of the figure.

References
References should be numbered in order of citation in the text, following the American Medical Association guidelines for references. The standard journal abbreviations from *Index Medicus* should be followed. Numbered references to personal communications, unpublished data, and manuscripts either “in preparation” or “submitted for publication” are unacceptable.

Drug Names
Generic names generally should be used. When proprietary brands are used in research, include the brand name in parentheses in the Methods section.

Units of Measure
Authors should express all measurements in conventional units, with Systeme International (SI) units given in parentheses throughout the text. Figures and tables should use conventional units, with conversion factors given in legends or footnotes.

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