Risk of Lyme disease: perceptions of residents of a Lone Star tick-infested community

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Background Lone Star ticks (*Amblyomma americanum*) have been suggested as a vector of the agent of Lyme disease (*Borrelia burgdorferi* sensu lato) in the USA, based on associations with an infection manifesting mainly as erythema migrans. In laboratory experiments, however, they failed to transmit *B. burgdorferi* sensu stricto.
Methods In this study, carried out from 1994 to 1996, we determined the seroprevalences of *B.burgdorferi* (1.2%), *Ehrlichia chaffeensis* (7%), *E. phagocytophila* (0%), *Rickettsia rickettsii* (0%), *R. typhi* (0%), *Coxiella burneti* (0%), *Francisella tularensis* (0%), and *Babesia microti* (0%) by standard serological methods for 325 residents (97% of the total population) of Gibson Island, coastal Maryland, USA, where 15% of the residents reported having had Lyme disease within a recent 5-year span.
Findings Of the 167 seronegative individuals who were followed up prospectively for 235 person-years of observation, only 2 (0.85%) seroconverted for *B. burgdorferi*. Of 1556 ticks submitted from residents, 95% were identified as Lone Star ticks; only 3% were deer ticks (*Ixodes dammini*), the main American vector of Lyme disease. *B. burgdorferi* s.s. infected 20% of host-seeking immature deer ticks, and borreliae (''*B. lonestari*'') were detected in 1–2% of Lone Star ticks. Erythema migrans was noted in 65% of self-reports of Lyme disease, but many such reports indicated that the rash was present while the tick was still attached, suggesting a reaction to the bite itself rather than true Lyme disease. Sera from individuals reporting Lyme disease generally failed to react to *B. burgdorferi* or any other pathogen antigens.
Conclusion The residents of Gibson Island had an exaggerated perception of the risk of Lyme disease because they were intensely infested with an aggressively human-biting and irritating nonvector tick. In addition, a Lyme disease mimic of undescribed etiology (named Masters' disease) seems to be associated with Lone Star ticks, and may confound Lyme disease surveillance. The epidemiological and entomological approach used in this study might fruitfully be applied wherever newly emergent tickborne zoonoses have been discovered.

Keywords Lyme disease/epidemiology; Ticks/pathogenicity; Tick-borne diseases/epidemiology; Borrelia burgdorferi/pathogenicity; Risk factors; Seroepidemiologic studies; United States (*source: MeSH*).

Mots clés Lyme, Maladie/épidémiologie; Tique/pathogénicité; Tiques, Maladies/épidémiologie; Borrelia burgdorferi/pathogénicité; Facteur risque; Etude séro-épidémiologique; Etats-Unis d'Amérique (*source: INSERM*).

Palabras clave Enfermedad de Lyme/epidemiología; Garrapatas/patogenicidad; Enfermedades por picaduras de garrapatas/epidemiología; Borrelia burgdorferi/patogenicidad; Factores de riesgo; Estudios seroepidemiológicos; Estados Unidos (*source: BIREME*).

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Voir page 923 le résumé en français. En la página 924 figura un resumen en español.

Introduction

The health burden of Lyme disease (an infection caused by *Borrelia burgdorferi*) in the eastern US is largely due to its tendency to occur in focal outbreaks. Indeed, the first 51 human infections that were recognized occurred in people residing in or near a place called Old Lyme (in coastal Connecticut), and involved 1 in 10 people living along only four roads (*1*). Similar clustered cases soon became evident in other sites in north-eastern USA. The seroprevalence ranges from 5% to 25% in communities located on Fire Island (on Long Island, New York), on Great Island (on Cape Cod in Massachusetts), in Ipswich (north of Boston, Massachusetts) and on Block Island (off the Rhode Island coast) (*2–5*). Representative incidences of Lyme disease in north-eastern USA during the two-year phase-III Lyme disease vaccine trials were estimated at 1.0–1.5% using stringent surveillance criteria (*6, 7*), but certain sites recorded a higher incidence (1.9–2.1%) (*8*).

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In the USA, cases of Lyme disease appear to cluster only as far south as Maryland, although virtually all states have reported some cases. In a summer camp in Kent County (in the north-eastern part of Maryland), for example, the sera of 16% of 51 employees reacted against *B. burgdorferi* antigen (*9*). In contrast, no such evidence of infection was discovered in a group of outdoor workers who had intense exposure to ticks on Assateague Island (in the south-eastern corner of Maryland) (*10*). In 1993, however, we learned of a residential site in central Maryland where the risk of Lyme disease appeared to be as intense as in the north-east of the state. The residents of this community, which included an experienced epidemiologist, provided a survey-based informal report that suggested a 12% incidence for physician-diagnosed Lyme disease during both the 1991 and 1992 summers. To the best of our knowledge, this site would represent the southern-most focus of intensely prevalent Lyme disease in the USA.

It may be that the clustered pattern of intense risk of Lyme disease that burdens the residents of many communities located in the north-eastern USA extends south to central Maryland. In such southerly sites, ticks other than those serving as vectors for Lyme disease (*Ixodes* spp.) more commonly infest people. Comprehensive epidemiological studies have not been carried out to determine whether dog ticks (*Dermacentor variabilis*) or Lone Star ticks (*Amblyomma americanum*) are associated with the risk of Lyme disease. To evaluate this possibility, we investigated the local risk of infection in an island community in central Maryland. In particular, we recorded the incidence of disease episodes reported by residents of the study site, estimated the frequency of human infection by a prospective serosurvey, and evaluated the risk by entomological means.

Materials and methods

Study site

Gibson Island in Maryland was chosen for this analysis because its residents anecdotally reported a disproportionate number of physician-diagnosed cases of Lyme disease and their intense annoyance due to ticks. The island site covers 380 ha and is linked to the western shore of Chesapeake Bay by a causeway, which is located about 25 km south-east of Baltimore. A total of 335 year-round residents lived there during the 1990 US census. The community comprises residents of higher than average socioeconomic and educational status. The oak forest that dominates the island is broken by several meadows. A dense understory of plants is mainly made up of kudzu (*Pueralia thumbergiana*) and bitter-sweet (*Celastrus scandens*). White-tailed deer (*Odocoileus virginianus*) are numerous; about 80 deer were identified by an aerial survey conducted in 1994 by the Maryland Department of Natural Resources. Other abundant animals include white-footed mice (*Peromyscus leucopus*), cottontail rabbits (*Sylvilagus floridanus*), grey squirrels (*Sciurus carolinensis*), and opossums (*Didelphis marsupialis*).

Serosurvey and questionnaire

Serum samples were taken cross sectionally from the residents of Gibson Island (with their free and informed consent) during the spring and fall of 1994 and during the fall of 1995 and 1996. Residents were asked, by means of a questionnaire, to describe their recent experiences with Lyme disease and ticks. They were also asked to describe the duration of their residence on the island, the nature of their outdoor activities, and any pets that they might own.

Serological testing

Serum samples were tested for evidence of infection by the agents of Lyme disease (*B. burgdorferi*), human monocytic ehrlichiosis (*Ehrlichia chaffeensis*), human granulocytic ehrlichiosis (*Ehrlichia phagocytophila*), Rocky Mountain spotted fever (*Rickettsia rickettsii*), murine typhus (*Rickettsia typhi*), Q fever (*Coxiella burneti*), tularaemia (*Francisella tularensis*) and babesiosis (*Babesia microti*). IgG antibodies against antigens of *E. chaffeensis, E. phagocytophila, R. rickettsii, R. typhi, C. burneti,* and *B. microti* were tested by indirect fluorescent antibody assays (IFA), as previously described (*11–14*). The serum samples were screened against *F. tularensis* by an agglutination assay, according to the manufacturer's recommendations (Difco Laboratories, Detroit, MI, USA).

IgG antibodies against the Lyme disease spirochaete were initially detected by enzyme-linked immunosorbent assay (ELISA) using antigens of the N40 strain of *B. burgdorferi* sensu stricto (*15*). Briefly, low-passage wildtype N40 was harvested at log-phase growth, washed three times in Hanks' balanced salt solution (HBSS), and resuspended in 20 times the original culture volume in HBSS. The washed spirochaetes were sonicated on ice until darkfield microscopy indicated that >85% of the organisms had been disrupted. After centrifugation to remove fragments and whole spirochaetes, the soluble extract was aliquoted and stored at –20 °C. Microtitre plates (Immulon 4, Dynex, Chantilly, VA, USA) were coated with 250 μg of antigen per well and allowed to dry. The plates were blocked with 5% donor horse serum, 1% dextran sulfate in PBS-Tween 20. Sera were diluted in this blocking buffer at 1:100, added to wells in triplicate, and incubated for 1 hour. Bound antibody was detected by the addition of alkaline phosphatase-conjugated goat antihuman IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA), as recommended by the manufacturer. A *p*-nitrophenyl phosphate substrate in diethanolamine buffer was used to visualize bound secondary antibody when the reactions were terminated at 30 minutes, and read promptly at $\lambda = 405$ nm. Negative control sera ($n = 6$), taken from laboratory staff members with no history of Lyme disease, were analysed on each plate. A serum sample was considered reactive if its mean absorbance

exceeded 3 standard deviations of the mean of the 18 (6 × 3) negative control wells, calculated for each microtitre plate.

All sera that were reactive or borderline by ELISA were characterized further by an immunoblotting procedure (*16*). Washed N40 spirochaetes were resuspended in SDS sample buffer (62.5 mmol/l Tris-Cl, pH 6.8, 2% sodium dodecyl sulfate, 50 mmol/l dithiothreitol, 10% glycerol, and 0.004% bromphenol blue), and were boiled for 5 minutes. Approximately 10^7 spirochaetes were loaded onto a 10% polyacrylamide gel (8.4 cm × 10 cm × 0.75 mm, with stacking gel), electrophoresed, and electrotransferred to nitrocellulose membrane (MSI, Westboro, MA, USA). For immunostaining, the membranes were blocked with 5% low-fat milk in Tris-buffered saline, pH 7.2, with 0.05% Tween 20. Sera were diluted (1:100) in this blocking buffer, and applied to the blot using a Miniblot apparatus (Immunetics, Cambridge, MA, USA). Bound antibody was detected using alkaline phosphatase-conjugated goat antihuman IgG (KPL), and visualized with bromochloroindolyl phosphate substrate with nitro blue tetrazolium chromophore (Amresco, Solon, OH, USA). The immunoblots were developed with the positive control serum (physician-confirmed, human Lyme disease serum from a Massachusetts site) until a standard control density was observed in its bands; there was no background staining with the negative control sera. Immunoblots were interpreted according to the criteria recommended by the Centers for Disease Control and Prevention (CDC) (*17*).

Entomological indices of risk

Ticks were sampled during the month of June in the years 1994–96 at a series of sites on Gibson Island by dragging a piece of flannel cloth over the surrounding vegetation. Field-derived deer ticks (*Ixodes dammini*) were screened for the presence of Lyme disease spirochaetes by polymer chain reaction (PCR). DNA was prepared from each tick by a chloroform–phenol extraction protocol, as previously described (*18*). Individual tick preparations were then screened by PCR using the *Borrelia*-specific primers, FLA-1/FLA-3; ticks containing *Borrelia* DNA were further analysed by means of the *B. burgdorferi*-specific primers, OspA2/OspA4 (*19*). Observations on non-infected, laboratory-reared deer ticks served as negative controls and were included with every batch of samples analysed by PCR. Extractions, reaction setup, and amplicon detection tasks were performed in different rooms with procedure-specific pipettors and reagents.

Lone Star ticks were analysed for evidence of infection during 1994 and 1995 (*20, 21*) by microscopy. Briefly, haemolymph samples from individual ticks were obtained from a stump of a leg and vitally examined by darkfield microscopy at × 400, and by staining dried preparations with Giemsa stain. In addition, gut samples were removed by dissection and analysed by IFA using a high-titred polyclonal rabbit antiserum to *B. burgdorferi* sensu lato.

Tick–human contact

The residents of Gibson Island helped us to estimate the frequency of tick–human contact by saving the ticks they found on their bodies, taping them on index cards, and submitting them to us for identification. The subject's name, the date, the anatomical site of tick attachment, and any subsequent signs of illness were noted on the "tick cards". The species and instar of all ticks were identified.

Data analysis

The incidence of Lyme disease was determined by dividing the number of newly seroreactive or symptomatic cases by the "total person-years of observation". This parameter is defined as the cumulative number of years during which all the resident subjects (initially non-seroreactive) could become seroreactive against the agent of Lyme disease, or reported that they suffered symptoms of this infection during the course of the study.

Data were analysed by means of χ^2 tests with Yates' continuity correction and Fisher's exact tests using INSTAT software (version 1.13), and were considered significant at $P < 0.05$. To test the association between potential risk factors and a history of Lyme disease, we applied bivariate and multivariate logistic regression models. All variables were initially analysed by bivariate analysis. Variables with associated $P < 0.20$ were added individually in a multiple logistic regression model using a stepwise model-fitting procedure (*22*). We sought combinations that increased the predictive value of the model or changed the point estimates of variables by more than 20%. Logistic regression analysis was performed using STATA software (version 5.0).

Results

Compliance of study population

Of an estimated 335 people who lived on Gibson Island, 97% participated in at least one of our serosurveys, conducted between 1994 and 1996, and 74% completed the questionnaires. A total of 167 initially seronegative residents were followed up prospectively for 235.3 person-years of observation. The residents were highly motivated and supportive of our research efforts.

Frequency of physician diagnosis

To estimate the perceived risk of human Lyme disease on Gibson Island, we asked each participating resident to describe any previous experience with this condition. Lyme disease was said to have been first diagnosed by a physician in 1988 on Gibson Island (Fig. 1). The annual reported incidence was greatest in 1992 and 1993 and declined thereafter. All together, 15.4% (95% confidence interval (CI) = 10.8–20) of the residents reported one or more episodes of physician-diagnosed Lyme disease between 1988 and 1996. Reporting bias may have

influenced this estimate of the prevalence inasmuch as individuals with a history of Lyme disease might have been more likely to complete the questionnaires. However, the self-reported prevalence on Gibson Island seems consistent with those for long-established north-eastern US sites of intense zoonotic Lyme transmission.

Reported signs and symptoms associated with Lyme disease

We asked the Gibson Island residents who reported to us that they had been diagnosed (within the preceding 8 years) by a physician as having Lyme disease to describe their symptoms. The clinical spectrum of Lyme disease in these subjects included as many as 12 different symptoms (Table 1). More than half experienced a rash, about a third experienced fever, and a quarter of them reported arthritis. Although these anecdotal reports could not serve as the primary data for reporting cases to the public health authorities according to CDC recommendations (*23*), they serve as an index of the community's perceptions of Lyme disease prevalence.

Association of diagnosed Lyme disease subjects with ticks

Residents reporting a history of Lyme disease were significantly more likely to garden, experience more than one tick bite per week, use personal protection measures, or reside in the study site for more than five consecutive summers (Table 2). Using multivariate logistic regression, we determined that "number of summers" spent in the summer site, "avoidance of brush", and "more than one tick bite per week" were statistically significant variables that were associated with a history of having been diagnosed with Lyme disease.

Serological analyses

To determine how frequently the residents of Gibson Island may be exposed to the agent of Lyme disease, we recorded seroconversion in a cohort of the population sampled during 1994 through 1996. Although the sera of 20.9% (95% CI = 15.8–26.8) of participants seroreacted against spirochaete antigen by IgG ELISA, only 1.2% (95% CI = 0.3–3.1) were confirmed for *B. burgdorferi* infection by immunoblotting. Of the four seropositive subjects in this sample, only two seroconverted during the course of the study, i.e a seroconversion rate of 0.85 (95% CI = 0.1–3.1) per 100 person-years observation. Of 37 residents who reported a history of Lyme disease, the sera of only three (8.1%) reacted against *B. burgdorferi* antigen by IgG immunoblotting. One additional seroreactive subject reported no symptoms consistent with this disease. The relative absence of specific seroreactivity against *B. burgdorferi* on Gibson Island seems incongruent with the community's perception of risk for infection to the agent of Lyme disease. However, the 0.85% incidence of Lyme disease, as measured by seroconversion during the study, is consistent with estimates of exposure within well-characterized northern communities in the USA.

To determine whether other tick-borne pathogens may have caused the reported episodes of physician-diagnosed Lyme disease, we screened the sera of affected residents against a battery of selected antigens. None reacted by IFA against the agents of babesiosis, granulocytic ehrlichiosis, Rocky Mountain spotted fever, and Q fever, and none reacted in an agglutination assay against tularaemia reagents. After finding that two out of 37 subjects reporting a history of Lyme disease seroreacted against *E. chaffeensis* antigen, we screened the entire study serum bank and found that 7% of residents seroreacted against the agent of monocytic ehrlichiosis. Seroreactivity against this agent did not correlate with a history of Lyme disease (odds ratio (OR) = 0.76, 95% CI = 0.17–3.4). The reports of the Gibson Island residents of episodes of Lyme disease cannot be attributed to exposure to any of the other common tick-borne pathogens that were tested.

Fig. 1. **No. of cases of Lyme disease reported by residents of Gibson Island, Maryland, USA, 1988–96**

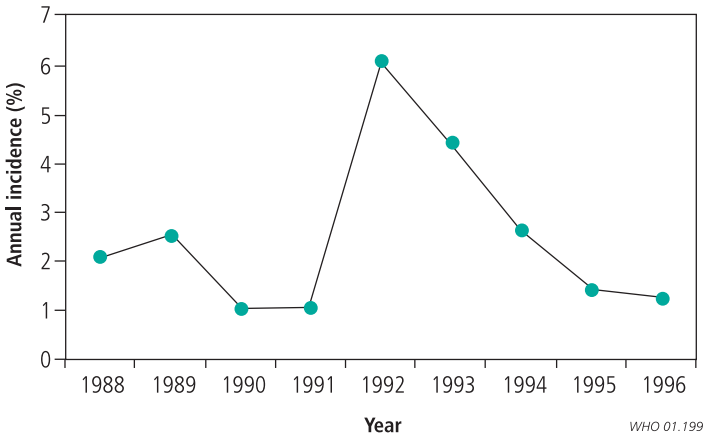

Table 1. **Distribution of symptoms experienced by 37 residents of Gibson Island, Maryland, USA, with a history of physician-diagnosed Lyme disease**

Symptom	% of residents (*n* = 37)
Rash	64.9
Muscle aches	35.1
Fever	32.4
Arthritis	24.3
Fatigue	18.9
Swelling of joints	5.4
Severe headaches	5.4
Night sweats	5.4
Facial paralysis	5.4
Visual disturbances	2.7
Stiff neck	2.7
Lymphoedema	2.7

Table 2. **Characteristics of residents of Gibson Island, Maryland, USA, who experienced prior physician-diagnosed episodes of Lyme disease compared to residents who reported no such diagnosis**

Variable	Crude odds ratio	Adjusted odds ratio[a]
Sex		
Males	0.6 (0.3–1.3)[b]	NA[c]
Age		
>40 years	2.5 (0.8–7.4)	NA
No. of summers on the island		
>5	14.6 (1.9–109)	14.3 (1.9–110)
Tick exposure		
>1 tick bite/week	2.1 (1.0–4.3)	2.3 (1.0–5.1)
Tick bite within last 2 months	1.1 (0.7–1.7)	NA
Ticks in yard	0.7 (0.4–1.2)	NA
Outdoor activities		
Gardening	3.4 (1.3–9.5)	2.6 (0.9–7.4)
Hiking	1.1 (0.5–2.3)	NA
Bird watching	1.7 (0.6–4.5)	NA
Golf	0.8 (0.4–1.7)	NA
Protection measures		
Repellents	1.6 (0.8–3.3)	NA
Long pants	1.6 (0.8–3.3)	NA
Avoiding the brush	3.8 (1.8–8.4)	4.6 (2.0–10.7)
Tick checks	3 (1.1–8.1)	NA
Pet ownership		
Cats	0.4 (0.1–1.7)	NA
Dogs	1.1 (0.5–2.3)	NA

[a] Adjusted for the number of summers on the island, more than 1 tick bite per week, gardening, and avoidance of brush.

[b] Figures in parentheses are 95% confidence intervals.

[c] NA = not applicable.

Entomological estimates of risk

To determine how frequently the residents of Gibson Island might be exposed to vector ticks infected by the agent of Lyme disease, we derived an entomological inoculation index. The relative questing density of vector ticks was estimated by flagging vegetation located near human habitations during the month of June. About 20–40 nymphal deer ticks[a] were captured per hour (Table 3). The presence of amplifiable *B. burgdorferi* DNA in a sample of these ticks was tested by PCR. Spirochaetal infection was evident in about 20% of these ticks when screened with PCR primers targeting a highly conserved region of the flagellin gene. Of these ticks, 94% reacted with OspA2 and OspA4 primers specific to *B. burgdorferi* sensu stricto (data not shown). Although the proportion of spirochaete-infected ticks increased over the course of the 3-year sampling period, the trend was not significant (χ^2 test for linear trend = 2.47, $P = 0.12$).

Lone Star ticks contained spirochaetes that are closely related to *B. theileri*, the agent of bovine borreliosis. During 1994, 2 out of 388 and, in 1995, 5 out of 297 host-seeking nymph and adult Lone Star ticks were infected (*20, 21*); none of these ticks contained *B. burgdorferi*.

We compared the inoculation indices for nymphal deer ticks and nymphal Lone Star ticks during 1994–95 (Table 4 and Table 5). The inoculation index was expressed as the product of the questing density of ticks and their prevalence of spirochaetal infection. On average, about 3.6 spirochaete-infected deer ticks, and 3.0 spirochaete-infected Lone Star ticks were collected per hour of sampling We conclude that the residents of Gibson Island encounter as many spirochaete-infected deer ticks as they do infected Lone Star ticks, but that the uninfected Lone Star ticks were an order of magnitude more likely to infest people than uninfected deer ticks.

Tick–human contact

To determine which ticks parasitize Gibson Island residents and how many of these are vector deer ticks, we identified all the ticks submitted to us during the course of our survey. Lone Star ticks (*A. americanum*) were the most abundant, accounting for 95% of the 1556 ticks submitted (Table 6). Only 3% were deer ticks, and the rest dog ticks (*D. variabilis*) and rabbit ticks (*I. dentatus*). The Lone Star ticks parasitize residents of our study site about 30 times more often than do deer ticks.

Residents reported frequent exposure to ticks, with 79% experiencing one or more tick bites "within the last 2 months". Of these, 42% were unable to distinguish between these kinds of ticks, and 34% stated that they had been bitten by at least one deer tick. Although Lone Star ticks were the most common human-biting tick, only 25% of respondents reported at least one bite from Lone Star ticks. Of 98 residents who reported a rash at the site of the tick bite, 53% recalled a rash when the tick was still attached — as such, this is more consistent with a cutaneous reaction to tick bites than with erythema migrans. We conclude that the residents frequently confused Lone Star ticks with deer ticks and that many of the tick-associated rashes may not have been true erythema migrans.

Of 152 residents reporting a recent tick bite ("within the last 2 months"), 20 had consulted a physician. Of these 20 individuals seeking medical care, 13 were prescribed antibiotics. A total of 54 ticks were submitted to us by these 20 patients, and 98% were identified as Lone Star ticks. We conclude that infestation with Lone Star ticks frequently prompted residents to seek medical assistance and that such visits frequently led to antibiotic treatment.

[a] By sequencing analysis of 16S mitochondrial DNA (*24, 25*), we determined that a sample of 12 deer ticks collected on Gibson Island comprised haplotypes associated with populations of ticks that we continue to refer to as *I. dammini* (*26*), although most workers now use the name *I. scapularis* to designate the Lyme disease vector (*27*).

Effect of reassurance on the frequency of Lyme disease diagnoses

After the 1994 transmission season, we informed the residents of the Island that few of their sera reacted with the agent of Lyme disease, and that many Lone Star ticks (but few vector deer ticks) fed upon them. We then distributed questionnaires to record the frequency of subsequent resident-reported Lyme disease diagnoses. The average annual self-reported incidence during 1992 through 1994 was 4% (95% CI = 2.6–5.8), compared to 1.3% (95% CI = 0.3–3.9) during 1995 through 1996. Only 7% (95% CI = 0.9–24.3) of resident-reported diagnoses of Lyme disease recorded during 1992–94 were confirmed serologically; 1 out of 3 reported cases was seroreactive during 1995–96. Overdiagnosis of Lyme disease among the Gibson Island residents appears to have resulted from an exaggerated perception of the risk, and public education may have helped to increase the accuracy of diagnoses.

Discussion

Our findings indicate that the risk of Lyme disease perceived on Gibson Island at the beginning of our study is exaggerated. Although this condition had previously been diagnosed in 37 of the island's residents, only three of these diagnoses could be confirmed serologically by immunoblot. In contrast, similar serological tests confirmed such diagnoses in almost half of a cohort of Connecticut residents (*28*). We recognize that serological tests may fail to confirm infection because prompt administration of antibiotics may abort the production of specific antibody (*29*, but see *30*). Perhaps the residents on Gibson Island sought medical attention more promptly than do others, thereby leading us to underestimate the serological exposure to Lyme disease antigen. The relative absence of asymptomatic seroconversions in our study population, however, suggests that this explanation is unlikely. The ratio of silent:symptomatic infections was about 1:4 during one of the phase-III Lyme disease vaccine trials (*7*). Because nearly 40 residents reported symptomatic infection (mainly a rash similar or identical to erythema migrans), we would expect to have found 8 seropositive individuals with no history of rash. Such asymptomatic infections would seroreact regardless of the health-seeking behaviour of our study population. Moreover, many of our Gibson Island subjects reported arthritis, a sequela that is usually accompanied by a strong antibody response (*31*). The individuals reporting recent arthritis would continue to be seroreactive if their symptoms had been due to *B. burgdorferi* infection. Taken together, this absence of specific seroreactivity among the residents of our study site suggests that the agent of Lyme disease did not cause many of the illnesses the residents and their physicians attributed to this pathogen.

Table 3. **Questing density of nymphal deer ticks infected by the agent of Lyme disease on Gibson Island, Maryland, USA, during the month of June, 1994–96**

Year	Flag sampling		Spirochaete DNA amplified		
	Total number of hours	No. of ticks per hour	No. of ticks tested	% of ticks infected	No. of infected ticks per hour
1994	3.2	23.8	47	10.6	2.5 (0.8–5.9)[a]
1995	6.8	32.5	100	18	5.8 (3.5–9.2)
1996	4.3	43.7	48	22.9	10 (5.0–17.9)
Total	**14.3**	**33.9**	**195**	**17.4**	**5.9 (4.1–8.3)**

[a] Figures in parentheses are 95% confidence intervals.

Table 4. **Entomological inoculation index (risk index) for Lyme disease and Lone Star tick spirochaetes, Gibson Island, Maryland, USA, 1994–95**

	Lyme disease spirochaete	Lone Star spirochaete
Nymphs/minute	0.55	5.41
Proportion infected	0.11	0.01
Risk index	0.06	0.05

Table 5. **Comparison of entomological risk for spirochaete-infected Lone Star ticks and deer ticks, Gibson Island, Maryland, 1994–95**

	Deer tick nymph	Lone Star nymph
Prevalence of infection	0.11	0.01
Proportion of questing ticks	0.06	0.76
Proportion of ticks on people	0.02	0.49

Table 6. **Diversity of 1556 submitted ticks that contacted residents of Gibson Island, Maryland, USA, 1994–96**

Species	Stage	% of submitted ticks
Amblyomma americanum	Larvae	9.5
	Nymph	49.8
	Adult	35.6
Ixodes dammini	Nymph	2.3
	Adult	1.0
Dermacentor variabilis	Adult	1.2
Ixodes dentatus	Nymph	0.5

Entomological evidence of Lyme disease on Gibson Island

The dearth of convincingly demonstrated cases of human Lyme disease on Gibson Island is consistent with concurrent entomological observations. The estimated entomological inoculation index (*32*) of 3 or 4 infected deer ticks per hour on the island is less than a fifth of that for the better characterized north-eastern sites. Residents of Gibson Island, therefore, are at some risk of acquiring Lyme disease, but this risk is not consistent with the community's perceptions. Interestingly, our entomological observations suggest that the force of Lyme disease spirochaete transmission may be intensifying on the island. The estimated density of spirochaete-infected deer ticks increased twofold each year over our 3-year sampling period. This apparent trend of increasing entomological risk is paradoxically inversely correlated with the incidence of self-reported physician-diagnosed Lyme disease cases. The observed decline of physician-diagnosed cases of Lyme disease from 1994 to 1996 could not be attributed to a corresponding decrease in the force of zoonotic transmission.

Risk factors for Lyme disease on Gibson Island

Analysis of the questionnaires for the risk factors that may be associated with self-reported Lyme disease proved to be paradoxical in some instances. Although the risks are evident for gardening or residence within the site for more than 5 years (both suggesting great possible exposure to ticks), use of personal protection measures should imply less risk. We interpret these associations as suggesting that those individuals who were concerned enough about tick exposure to implement protection measures may have been more likely to visit a physician after a tick bite and suggest the diagnosis of Lyme disease, and/or report their history on our questionnaire.

Tickborne diseases and Gibson Island

The intense infestation of Lone Star ticks on Gibson Island may help suppress the risk of human Lyme disease. Lone Star ticks are not competent vectors for *B. burgdorferi* sensu stricto (*33, 34*), although their competence for other *B. burgdorferi* genospecies remains undescribed. These aggressively human-biting ticks seem more broadly distributed on the island than are deer ticks; they quest over a greater portion of the summer period; and they are far more common throughout the island's habitat types (*35*). The intense annoyance caused by this infestation of Lone Star ticks may have caused the residents of Gibson Island to avoid contact with habitats that might harbour any ticks, including deer ticks. Indeed, virtually all (85%) local residents informed us that they regularly protected themselves against ticks, and half (47%) of them assiduously avoided contact with vegetation. This behaviour results from the activity of nonvector ticks because virtually all (95%) ticks that attach to the Gibson Island residents are Lone Star ticks. In this sense, certain nonvector ticks may help prevent Lyme disease.

In addition to inducing the residents of Gibson Island to protect themselves from the bites of vector ticks, the intense infestation of Lone Star ticks may have induced patients to frequently suggest the possibility of Lyme disease to their physicians. Residents of the island seem to have misclassified these ticks as deer ticks and frequently misinterpreted the rash produced by their bite as the hallmark (erythema migrans) lesion of Lyme disease. Tick-induced lesions may become erythematous before the tick detaches from the skin and remain evident for some time after the tick is removed; perivascular inflammatory infiltrates may be observed in the dermis even four days after bites from Lone Star ticks (*36*). Erythema migrans, in contrast, begins to form several days after the tick is detached from the skin (*37*). Of those residents who reported a rash at the site of the tick bite, more than half recalled a rash when the tick was still attached, consistent with a local reaction to tick bite and not classical erythema migrans. Their attending physicians may also have been influenced by these misperceptions.

Similar Lyme-disease-like illnesses of unknown etiology have been associated with the bites of Lone Star ticks in Missouri and North Carolina (*38, 39*). This syndrome, termed Masters' disease (*40*) or southern-tick-associated rash-illness (STARI) (*41*), is characterized by an expanding erythema migrans rash from which no evidence of *B. burgdorferi* (organisms, antigen, or DNA) may be detected, accompanied by generally mild constitutional symptoms. Reactivity to *B. burgdorferi* antigens, as strictly defined by the Council of State and Territorial Epidemiologists/ Association of State and Territorial Public Health Directors (CSTE/ASTPHLD) recommendations for interpreting Lyme immunoblots, seems rare in sera from these patients (*38*). Although the Gibson Island cases reported a frequency of fever and muscle aches similar to those from Missouri, the symptoms of fatigue, headache, and stiff neck seemed less common. Nonetheless, we conclude that a syndrome characterized by erythema migrans and without confirmatory serological evidence of exposure to *B. burgdorferi* antigens — a clinical entity we consider distinct from Lyme disease and have referred to as Masters' disease — seems to commonly affect the residents of Gibson Island. Risk for *bona fide* Lyme disease, although present, is less intense and confounded by that for Masters' disease.

Although no evidence of infection by any of the common tick-borne pathogens was found, the possibility of a previously unrecognized pathogen remains. Lone Star ticks harbour a spirochaete, *B lonestari,* closely related to relapsing fever spirochaetes and to *B. theileri* but as yet uncultivated (*21, 41*). On Gibson Island, about 1–2% of Lone Star ticks harbour such spirochaetes (*20, 21*). Perhaps the Lone Star tick spirochaete is responsible for the episodes of Masters' disease noted on Gibson Island. No specific serological assays are at present available

to directly assess prior exposure to the Lone Star tick spirochaete. Borreliae in general are highly cross-reactive (*42*), and although antigenic differences may affect immunoblot confirmation of infections by the diverse *B. burgdorferi* sensu lato (*43*), ELISA reactivities remain similar. In this study, seroreactivity against the Lyme disease spirochaete, as determined by ELISA, did not correlate with a reported history of Lyme disease (OR = 1.2, 95% CI = 0.5–3.1). Use of sonicated *B. hermsi* (HS-1 strain) or a local *B. burgdorferi* isolate (GIB-001, from *P. leucopus*) as ELISA antigen did not change the patterns of sample reactivity (data not shown). This absence of association with general borrelial seroreactivity suggests that the majority of illnesses attributed to Lyme disease on Gibson Island did not result from infection by *B. burgdorferi* sensu lato or by other *Borrelia* spp.

The intense concurrent clustering of cases of Lyme disease, human babesiosis, and human granulocytic ehrlichiosis in particular residential sites of the north-eastern US is not evident in Maryland. Only one apparent outbreak of Lyme disease has been reported, affecting 8 employees of a summer camp on the eastern shore of the Chesapeake Bay (*9*). Few residents of Gibson Island seem to have acquired Lyme disease that could be confirmed by serological means; nor did we find evidence of the other agents that are co-transmitted with the agent of Lyme disease further north in the USA. In New England, for example, the agent of human babesiosis had infected 11% of Lyme disease patients (*5*). Infection due to deer tick-associated pathogens is more intense and apparently more diverse in the north-eastern US than on Gibson Island.

The burgeoning deer population in much of the eastern and central US may increasingly promote public health burdens due to tickborne disease (*44, 45*). Deer serve as the main reproductive host for *I. dammini/I. scapularis*, as well as for *A. americanum*. Diverse guilds of microbes are associated with each kind of tick (*40*). Complex epidemiological situations are expected in sites where both kinds of ticks co-occur, such as in much of New Jersey (*46*). Residents may not accurately identify ticks. Lone Star tick bites may provoke a cutaneous reaction that may be misidentified as erythema migrans. True Lyme disease may be confused with Masters' disease, while human ehrlichiosis may comprise granulocytic or monocytic agents (*47*). The relative nonspecificity of the signs and symptoms of most of the tickborne zoonoses, in association with difficulties of confirmatory laboratory testing, poses a diagnostic challenge to physicians wishing to assign etiology. Similarly, determining the public health burden due to each tickborne infection will be difficult for American epidemiologists.

The wide distribution of Lyme disease and associated infections across the temperate zone, from North America through Eurasia, suggests that similar situations where etiology is confounded may emerge. The comprehensive epidemiological and entomological approach that we have described may provide a means of describing the correlates of risk for newly emergent tickborne zoonoses and, ultimately, facilitate the development of means for intervention. ■

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Résumé

Perception du risque de maladie de Lyme par les résidents d'une communauté infestée par des tiques étoilées américaines

Introduction La tique étoilée américaine (*Amblyomma americanum*) a été incriminée en tant que vecteur de l'agent de la maladie de Lyme (*Borrelia burgdorferi* sensu lato) aux Etats-Unis d'Amérique, sur la base d'associations avec une infection se manifestant principalement par un érythème chronique migrateur. Cependant, les tiques de cette espèce se sont révélées incapables de transmettre *B. burgdorferi* sensu stricto lors d'expériences de laboratoire.

Méthodes Lors de la présente étude, réalisée de 1994 à 1996, nous avons déterminé la séroprévalence de *B. burgdorferi* (1,2 %), *Ehrlichia chaffeensis* (7 %), *E. phagocytophila* (0 %), *Rickettsia rickettsii* (0 %), *R. typhi* (0 %), *Coxiella burneti* (0 %), *Francisella tularensis* (0 %) et *Babesia microti* (0 %) par des méthodes sérologiques classiques chez 325 résidents (97 % de la population totale) de Gibson Island, sur la côte du Maryland (Etats-Unis d'Amérique), où 15 % des résidents avaient déclaré avoir été atteints de maladie de Lyme au cours d'une récente période de cinq ans.

Résultats Parmi les 167 résidents séronégatifs, qui ont fait l'objet d'un suivi prospectif sur 235 personnes-années d'observation, seuls 2 (0,85 %) ont présenté une séroconversion vis-à-vis de *B. burgdorferi*. Sur 1556 ti-

ques apportées par les résidents, 95 % ont été identifiées comme tiques étoilées américaines (*Amblyomma americanum*) ; 3 % seulement étaient des tiques de l'espèce *Ixodes dammini* (tique du cerf), principal vecteur américain de la maladie de Lyme. *B. burgdorferi* s.s. était présente chez 20 % des jeunes tiques du cerf à la recherche d'un hôte, et d'autres *Borrelia* (« *B. lonestari* ») ont été détectées chez 1 à 2 % des tiques étoilées américaines. Un érythème chronique migrateur a été noté dans 65 % des cas chez les résidents ayant rapporté des épisodes de maladie de Lyme, mais un grand nombre de ces rapports mentionnaient la présence d'une éruption alors même que la tique étaient encore fixée à la peau, ce qui semble indiquer une réaction à la piqûre de tique plutôt qu'une maladie de Lyme proprement dite. En général, le sérum des personnes faisant état d'une maladie de Lyme ne réagissait ni avec *B. burgdorferi* ni avec des antigènes d'autres agents pathogènes.

Conclusion Les résidents de Gibson Island avaient une perception exagérée du risque de maladie de Lyme en raison d'une infestation massive de leur environnement par une tique piqueuse agressive et irritante, mais non vectrice. De plus, une affection simulant la maladie de Lyme, d'étiologie inconnue (appelée maladie de Masters) semble associée à la tique étoilée américaine et peut fausser les données de la surveillance. L'approche épidémiologique et entomologique adoptée dans cette étude pourrait être utilement mise en œuvre partout où l'on découvre l'émergence récente de zoonoses transmises par des tiques.

Resumen

Percepción del riesgo de enfermedad de Lyme entre los residentes de una comunidad infestada de garrapatas Lone-Star

Antecedentes A partir de las asociaciones establecidas con una infección que se manifiesta fundamentalmente en forma de eritema migrans, se ha señalado que las garrapatas Lone Star podrían actuar como vector del agente de la enfermedad de Lyme (*Borrelia burgdorferi* sensu lato) en los Estados Unidos. Sin embargo, en experimentos de laboratorio no se observó la transmisión de *B. burgdorferi* sensu stricto por dichas garrapatas.

Métodos En este estudio, llevado a cabo entre 1994 y 1996, determinamos la seroprevalencia de *B. burgdorferi* (1,2%), *Ehrlichia chaffeensis* (7%), *E. phagocytophila* (0%), *Rickettsia rickettsii* (0%), *R. typhi* (0%), *Coxiella burneti* (0%), *Francisella tularensis* (0%), y *Babesia microti* (0%) mediante métodos serológicos para 325 residentes (97% de la población total) de la isla de Gibson, en la costa de Maryland (EE.UU.), donde el 15% de los residentes refirieron haber padecido la enfermedad de Lyme en los últimos 5 años.

Resultados De los 167 individuos seronegativos a los que se sometió a un seguimiento prospectivo durante 235 años-persona, sólo 2 (0,85%) pasaron a ser seropositivos para *B. burgdorferi*. De las 1556 garrapatas enviadas por los residentes, el 95% resultaron ser del tipo Lone Star (*Amblyomma americanum*), y sólo 3% eran garrapatas del ciervo (*Ixodes dammini*), el principal vector de la enfermedad de Lyme en Norteamérica. *B. burgdorferi* s.s. infectaba al 20% de las garrapatas del ciervo inmaduras que necesitan huésped, y se detectó borreliae (''*B. lonestari*'') en un 1%–2% de las garrapatas Lone Star. Se refirió la presencia de eritema migrans en un 65% de los casos de enfermedad de Lyme autonotificados, pero muchas de esas veces el exantema coincidía con la adhesión de la garrapata, lo que permite interpretarlo más como una reacción a la picadura que como manifestación de la verdadera enfermedad de Lyme. Los sueros de los individuos que declararon signos de la enfermedad de Lyme no mostraron por lo general reacción ni a *B. burgdorferi* ni a antígenos de otros agentes patógenos.

Conclusión Los residentes de la isla de Gibson sobrestimaban el riesgo de enfermedad de Lyme debido a los frecuentes casos de infestación por una garrapata no vectorial de picadura agresiva y efectos irritantes. Además, existe un cuadro clínico de etiología no determinada parecido a la enfermedad de Lyme (conocido como enfermedad de Masters) que se asocia a las garrapatas Lone Star y que puede interferir en la vigilancia de la enfermedad. El enfoque epidemiológico y entomológico empleado en este estudio podría aplicarse de forma fructífera en todos aquellos casos en que se han descubierto nuevas zoonosis emergentes transmitidas por garrapatas.

References

1. **Steere AC et al.** An epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. *Arthritis and Rheumatism*, 1977, **20**: 7–17.
2. **Hanrahan JP et al.** Incidence and cumulative frequency of endemic Lyme disease in a community. *Journal of Infectious Diseases*, 1984, **150**: 489–496.
3. **Steere AC et al.** Longitudinal assessment of the clinical and epidemiological features of Lyme disease in a defined population. *Journal of Infectious Diseases*, 1986, **154**: 295–300.
4. **Lastavica CC et al.** Rapid emergence of a focal epidemic of Lyme disease in coastal Massachusetts. *New England Journal of Medicine*, 1989, **320**: 133–137.
5. **Krause PJ et al.** Concurrent Lyme disease and babesiosis: evidence for increased severity and duration of illness. *Journal of the American Medical Association*, 1996, **275**: 1657–1660.
6. **Sigal LH et al.** A vaccine consisting of recombinant *Borrelia burgdorferi* outer surface protein A to prevent Lyme disease. *New England Journal of Medicine*, 1998, **339**: 216–222.
7. **Steere AC et al.** Vaccination against Lyme disease with recombinant *Borrelia burgdorferi* outer surface lipoprotein A with adjuvant. *New England Journal of Medicine*, 1998, **339**: 209–215.

8. **Wormser GP et al.** Efficacy of an OspA vaccine preparation for prevention of Lyme disease in New York State. *Infection*, 1998, **26**: 208–211.
9. **Strickland GT et al.** Cluster of Lyme disease cases at a summer camp in Kent County, Maryland. *Emerging Infectious Diseases*, 1996, **2**: 44–46.
10. **Parrott C et al.** Lyme disease in outdoor workers on Assateague Island: high tick exposure but low disease risk. *Maryland Medical Journal*, 1993, **42**: 165–168.
11. **Dawson JE et al.** Isolation and characterization of an *Ehrlichia* sp. from a patient diagnosed with human ehrlichiosis. *Journal of Clinical Microbiology*, 1991, **29**: 2741–2745.
12. **Nicholson WL et al.** An indirect immunofluorescence assay using a cell culture-derived antigen for detection of antibodies to the agent of human granulocytic ehrlichiosis. *Journal of Clinical Microbiology*, 1997, **35**: 1510–1516.
13. **Philip RN et al.** Microimmunofluorescence test for the serological study of Rocky Mountain spotted fever and typhus. *Journal of Clinical Microbiology*, 1976, **3**: 51–61.
14. **Krause PJ et al.** Diagnosis of babesiosis: evaluation of a serologic test for the detection of *Babesia microti* antibody. *Journal of Infectious Diseases*, 1994, **169**: 923–926.
15. **Telford SR III, Spielman A.** Enzootic transmission of the agent of Lyme disease in rabbits. *American Journal of Tropical Medicine and Hygiene*, 1989, **41**: 482–490.
16. **Rosa Brunet L et al.** Antibody response of the mouse reservoir of *Borrelia burgdorferi* in nature. *Infection and Immunology*, 1995, **63**: 3030–3036.
17. **Centers for Disease Control and Prevention.** Recommendations for test performance and interpretation from second national conference on serologic diagnosis of Lyme disease, 1995. *Morbidity and Mortality Weekly Report*, 1995, **44**: 590–591.
18. **Armstrong PM et al.** Diversity of *Babesia* infecting deer ticks (*Ixodes dammini*). *American Journal of Tropical Medicine and Hygiene*, 1998, **58**: 739–742.
19. **Persing DH et al.** Detection of *Borrelia burgdorferi* DNA in museum specimens of *Ixodes dammini* ticks. *Science*, 1990, **249**: 1420–1423.
20. **Armstrong P et al.** A new *Borrelia* infecting Lone Star ticks. *Lancet*, 1996, **347**: 67–68.
21. **Armstrong P et al.** Lone Star tick-infecting borreliae are most closely related to the agent of bovine borreliosis. *Journal of Clinical Microbiology*, 2001, **39**: 494–497.
22. **Hosmer DW, Lemeshow S.** Model-building strategies and methods for logistic regression. In: *Applied logistic regression.* New York, John Wiley & Sons, 1989: 82–134.
23. **Centers for Disease Control and Prevention.** Case definitions for infectious conditions under public health surveillance. *Morbidity and Mortality Weekly Report*, 1997, **RR10**: 20–21.
24. **Rich SM, Caporale DA, Telford SR III et al.** Distribution of the *Ixodes ricinus*-like ticks of eastern North America. *Proceedings of the National Academy of Sciences of the USA*, 1995, **92**: 6284–6288.
25. **Norris DE et al.** Population genetics of *Ixodes scapularis* based on mitochondrial 16S and 12S genes. *Journal of Medical Entomology*, 1996, **33**: 78–89.
26. **Telford SR III.** Use of *Ixodes dammini* is epidemiologically justified. *Emerging Infectious Diseases*, 1998, **4**: 132–133.
27. **Oliver JH et al.** Conspecificity of the ticks *Ixodes scapularis* and *I. dammini* (Acari: Ixodidae). *Journal of Medical Entomology*, 1993, **30**: 54–63.
28. **Feder HM et al.** Prospective assessment of Lyme disease in a school-aged population in Connecticut. *Journal of Infectious Diseases*, 1995, **171**: 1371–1374.
29. **Craft JE, Grodzicki RL, Steere AC.** Antibody response in Lyme disease: evaluation of diagnostic tests. *Journal of Infectious Diseases*, 1984, **149**: 789–795.
30. **Aguero-Rosenfeld ME et al.** Evolution of the serologic response to *Borrelia burgdorferi* in treated patients with culture confirmed erythema migrans. *Journal of Clinical Microbiology*, 1996, **34**: 1–9.
31. **Feder HM et al.** Persistence of serum antibodies to *Borrelia burgdorferi* in patients treated for Lyme disease. *Clinical Infectious Diseases*, 1992, **15**: 788–793.
32. **Piesman J et al.** Seasonal variation of risk of infection by *Borrelia burgdorferi* and *Babesia microti. American Journal of Epidemiology*, 1987, **126**: 1187–1189.
33. **Piesman J, Sinsky RJ.** Ability of *Ixodes scapularis*, *Dermacentor variabilis*, and *Amblyomma americanum* (Acari:Ixodidae) to acquire, maintain, and transmit Lyme disease spirochetes (*Borrelia burgdorferi*). *Journal of Medical Entomology*, 1988, **25**: 336–339.
34. **Piesman J, Happ CM.** Ability of the Lyme disease spirochete *Borrelia burgdorferi* to infect rodents and three species of human biting ticks (blacklegged tick, American dog tick, Lone Star tick) (Acari:Ixodidae). *Journal of Medical Entomology*, 1997, **34**: 451–456.
35. **Sonenshine DE, Levy GF.** The ecology of the Lone Star tick, *Amblyomma americanum*, in two contrasting habitats in Virginia. *Journal of Medical Entomology*, 1971, **8**: 623–635.
36. **Goldman L, Rockwell E, Richfield DF.** Histopathological studies on cutaneous reactions to the bites of various arthropods. *American Journal of Tropical Medicine and Hygiene*, 1952, **1** (3): 514–525.
37. **Steere AC et al.** The early clinical manifestations of Lyme disease. *Annals of Internal Medicine*, 1983, **99**: 76–82.
38. **Campbell GL et al.** Epidemiologic and diagnostic studies of patients with suspected early Lyme disease, Missouri, 1990–1993. *Journal of Infectious Diseases*, 1995, **172**: 470–480.
39. **Kirkland KB et al.** Erythema migrans-like rash illness at a camp in North Carolina, a new tick-borne disease? *Archives of Internal Medicine*, 1997,**157**: 2635–2641.
40. **Telford SR, Dawson JE, Halupka KC.** Emergence of tick-borne diseases. *Science and Medicine*, 1997, **4**: 24–33.
41. **Barbour AG et al.** Identification of an uncultivatable *Borrelia* species in the hard tick *Amblyomma americanum*: possible agent of a Lyme disease-like illness. *Journal of Infectious Diseases*, 1996, **173**: 403–409.
42. **Magnarelli LA, Anderson JF, Barbour AG.** Enzyme-linked immunosorbent assays for Lyme disease: reactivity of subunits of *Borrelia burgdorferi. Journal of Infectious Diseases*, 1989, **159**: 43–49.
43. **Dressler F, Ackermann R, Steere AC.** Antibody responses to the three genomic groups of *Borrelia burgdorferi* in European Lyme borreliosis. *Journal of Infectious Diseases*, 1994, **169**: 313–318.
44. **Spielman A et al.** Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. *Annual Review of Entomology*, 1985, **30**: 439–460.
45. **Spielman A, Telford SR III, Pollack RJ.** The origins and course of the outbreak of Lyme disease. In: Ginsburg H, ed., *Ecology and management of Lyme disease.* New Brunswick, NJ, Rutgers University Press, 1993: 83–96.
46. **Bowen GS et al.** A focus of Lyme disease in Monmouth County, New Jersey. *American Journal of Epidemiology*, 1984, **120**: 387–394.
47. **Telford SR III, Dawson JE.** Diagnosing ehrlichiosis. *Annals of Internal Medicine*, 1996, **124**: 854.