Duration of Tick Attachment as a Predictor of the Risk of Lyme Disease in an Area in which Lyme Disease Is Endemic

Sunil K. Sood, Mark B. Salzman,* Barbara J. B. Johnson, Christine M. Happ, Kevin Feig,* Lillian Carmody, Lorry G. Rubin, Eileen Hilton, and Joseph Piesman

Division of Infectious Diseases, Department of Pediatrics, Schneider Children's Hospital, and Department of Medicine, Long Island Jewish Medical Center and Albert Einstein College of Medicine, New Hyde Park, New York; Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado; Department of Preventive Medicine, State University of New York Health Sciences Center at Stony Brook, Stony Brook, New York

Animal studies have shown an exponential increase in the risk of *Borrelia burgdorferi* infection after 48–72 h of deer tick attachment. Persons with tick bites were prospectively studied to determine if those with prolonged tick attachment constitute a high-risk group for infection. Ticks were identified, measured for engorgement, and assayed by polymerase chain reaction (PCR) for *B. burgdorferi* DNA. Duration of attachment was determined from the scutal index of engorgement. Of 316 submissions, 229 were deer ticks; 14% were positive by PCR. Paired sera and an intact tick for determination of duration of attachment were available for 105 subjects (109 bites). There were 4 human cases (3.7% of bites) of *B. burgdorferi* infection. The incidence was significantly higher for duration of attachment ⩾72 h than for <72 h: 3 (20%) of 15 vs. 1 (1.1%) of 94 (P = .008; odds ratio, 23.3; 95% confidence interval, 2.2–242). PCR was an unreliable predictor of infection. Tick identification and measurement of engorgement can be used to identify a small, high-risk subset of persons who may benefit from antibiotic prophylaxis.

Lyme disease results from *Borrelia burgdorferi* infection, which is acquired after the bite of an *Ixodes* tick, chiefly *Ixodes scapularis* in the United States. Although symptomatic infection most commonly presents as the skin lesion erythema migrans, dissemination to other organs may occur hematogenously. Because such organ involvement can be associated with severe and occasionally persistent disease, consideration is often given to the administration of antibiotic prophylaxis following a bite.

The incidence of infection following an individual tick bite is low (1.2%–3%), even in areas in which Lyme disease is endemic [1–3]. Moreover, treatment of symptomatic disease is effective. The decision to use prophylaxis must therefore take into consideration the risk of adverse effects of the antibiotic used, such as photosensitivity with tetracyclines, the growing risk of antibiotic-resistant bacteria in the community, and the absence of a clear demonstration of efficacy of prophylactic treatment [3]. Cost-effectiveness analysis has been used in an attempt to determine risk cutoffs for empirical treatment of persons bitten by a deer tick [4]. It was concluded that a strategy of treating all persons was indicated in areas where the probability of infection was ⩾3.6%. However, precise data on local incidence of infection following a tick bite are lacking, and the practice of administering prophylactic therapy remains controversial [5]. We sought to prospectively measure the incidence of *B. burgdorferi* infection in untreated patients with a proven *I. scapularis* bite in an area in which Lyme disease is endemic and to identify a high-risk group for which prophylaxis may be indicated.

The duration of attachment of the tick is thought to be an important variable that influences the risk of infection [4, 6]. The spirochete resides and replicates in the midgut of the tick, and there is a delay between the onset of feeding and the appearance of infectious spirochetes in the tick saliva. Data from tick-feeding animal models indicate an exponential increase in spirochete transmission after 48–72 h of attachment [6, 7]. The duration of attachment can be calculated from an index of tick engorgement, termed the scutal index [8, 9]. We sought to determine whether prolonged duration of attachment correlates with an increased risk of infection in humans.

An additional variable that influences the risk of infection is the likelihood of the implicated deer tick to harbor *B. burgdorferi*. The rate of carriage of the spirochete in areas in which Lyme disease is endemic can vary widely (10%–100%). We assayed for presence of *B. burgdorferi* by the polymerase chain reaction (PCR) as part of our tick analysis.

Received 26 July 1996; revised 8 November 1996.

Presented in part: Society for Pediatric Research, annual meeting, Washington, DC, 3–6 May 1993 (abstract 1084); and Sixth International Conference on Lyme Borreliosis, Bologna, Italy, 19–22 June 1994 (abstract P018W).

Informed consent was obtained for the drawing of blood.

Reprints or correspondence: Dr. Sunil K. Sood, Schneider Children's Hospital, 269-01 76th Ave., New Hyde Park, NY 11040.

* Current affiliations: Kaiser Permanente Medical Center (West Los Angeles), Los Angeles (M.B.S.); Corporate Health Center, Trenton, New Jersey (K.F.).

The Journal of Infectious Diseases 1997; 175:996–9

0022–1899/97/7504–0044$01.00

Methods

Study design. The study was conducted in the greater New York metropolitan area, an area in which Lyme disease is endemic, from June 1992 through September 1993. The subjects were persons who reported being bitten by a deer tick in the preceding 72 h, submitted the tick, and agreed to paired serologic testing and to maintain follow-up contact. Persons who were receiving an antibiotic were excluded. Subjects reported their estimate of the length of time the tick was attached, and this was rounded to the nearest 12-h period. Subjects were examined if any rash occurred. Criteria for erythema migrans were a diameter of ⩾5 cm with at least partial central clearing. No antibiotics were administered unless the subject developed erythema migrans.

Serology and infection. Sera were obtained on the day of tick submission (baseline) and 4–6 weeks later (convalescent) and assayed as pairs. EIA was performed by the IgG/IgM FASTLYME method (BioWhittaker, Walkersville, MD). IgG immunoblot was performed as described [10], with baseline and convalescent sera assayed simultaneously on adjacent strips of the same blot, and interpreted by standard criteria [11]. An incident case of *B. burgdorferi* infection was defined as either the occurrence of erythema migrans between the time the tick bite occurred and the second visit, seroconversion by EIA and immunoblot, or both.

Tick analysis. Submitted specimens were identified, including species, sex, and stage (larva, nymph, adult; male and female), using standard taxonomic keys.

An ocular micrometer fitted to a Wild microscope was used to measure the body length (BL: the posterior edge of the basis capitulum to the posterior tip of the opisthosoma) and the maximum width of the scutum (scutal width, SW). The scutal index was computed as the ratio BL/SW. This measure of engorgement has been shown to correlate directly with the duration of attachment of *I. scapularis* nymphs and females, because of an exponential increase in body length during feeding and an inflexible scutum [9]. Separate exponential regression equations for nymphs and females were used to calculate duration of attachment in hours from the scutal index value for each tick. Ticks were divided into duration of attachment groups: <24, 24–48, 48–72, and ⩾72 h.

B. burgdorferi DNA in ticks was detected by polymerase chain reaction (PCR) that amplified a fragment of the flagellin gene, as described [12]. All DNA templates were prepared by phenol extraction of tick guts, ether washing, and ethanol precipitation. The nested primer protocol was adapted to a one-tube format, which minimizes amplicon contamination, and all sentinel control reactions were negative.

Statistical methods. Odds ratios and significance of differences between proportions were calculated by standard methods (Instat statistical package).

Results

Ticks. Three hundred sixteen specimens were submitted by 312 persons who reported a tick bite in the preceding 72 h; 303 were ticks. Two hundred twenty-nine (75.6%) were *I. scapularis* (the deer tick), 42 (13.9%) *Dermacentor variabilis* (American dog tick), 18 (5.94%) *Amblyomma americanum* (Lone star tick), 8 (2.64%) *Rhipicephalus sanguineus* (brown dog tick), 5 (1.65%) *Ixodes dentatus*, and 1 (0.33%) *Ixodes cookei*. The other specimens were identified as beetles (2), a crab louse (1), a louse nit (1), and artifacts (9), usually skin scabs or inanimate fragments. *I. scapularis* constituted 72.5% of submissions.

The scutal index was measured in 193 intact nymphal and female *I. scapularis.* Duration of attachment was <24 h in 122 ticks (63.2%), 24–48 h in 18 (9.3%), 48–72 h in 28 (14.5%), and ⩾72 h in 25 (13%).

PCR assay for *B. burgdorferi* DNA was performed on 227 *I. scapularis* ticks. The 32 positive ticks comprised 25 females (23.4% of 107), 5 nymphs (4.8% of 104), 1 larva (5.9% of 17), and the only male tick.

Incidence of infection in study subjects. The 229 *I. scapularis* were submitted by 225 subjects; 4 of these subjects had 2 simultaneous tick bites. Subjects with larvae were excluded from further analysis, as the larva is not considered a likely vector. One hundred fifteen subjects (119 bites) with nymphal or adult deer ticks completed paired serologic testing (figure 1). The scutal index could be measured in 109 intact nymphal or female ticks. Four subjects developed *B. burgdorferi* infection (3.7%) (table 1). One occurred in duration of attachment group <24 h and 3 in duration of attachment group ⩾72 h.

The incidence of *B. burgdorferi* infection according to type of tick bite can be seen in figure 1. Female and nymphal *I. scapularis* bites resulted in similar rates of infection (4% and 3.4%). However, the risk of infection was significantly higher from nymphal *I. scapularis* attached ⩾72 h when compared with all other *I. scapularis* bites (18% vs. 2%; $P = .05$; odds ratio [OR], 10.7; 95% confidence interval [CI], 1.4–85). For female *I. scapularis* attached ⩾72 h, when compared with all other *I. scapularis* bites, the risk was higher (25% vs. 2.9%), but this was not statistically significant ($P = .14$; OR, 11.3; 95% CI, 0.9–143.5). When data for female and nymph bites were combined, the incidence was significantly different between duration of attachment <72 h and duration of attachment ⩾72 h: 1 (1.1%) of 94 and 3 (20%) of 15, respectively ($P = .008$; OR, 23.3; 95% CI, 2.2–242).

Two nymphal ticks with duration of attachment ⩾72 h were PCR-positive and both bites resulted in infection (positive predictive value of a PCR-positive nymph with duration of attachment ⩾72 h = 100%). In contrast, both female ticks that resulted in infection were PCR-negative.

Although paired sera were not available for 106 subjects, none developed erythema migrans. Thus, with 225 subjects as the denominator, the incidence of infection was 1.8%, if additional possible asymptomatic seroconversions are excluded.

Accuracy of history for duration of attachment. Only 94 subjects (49% of those who submitted an intact tick) could estimate the duration of attachment within a 24-h range. This duration of attachment by history had a poor correlation with the duration of attachment obtained by scutal index measurement ($r^2 = .19$ for female *I. scapularis*, .41 for nymphal *I. scapularis*).

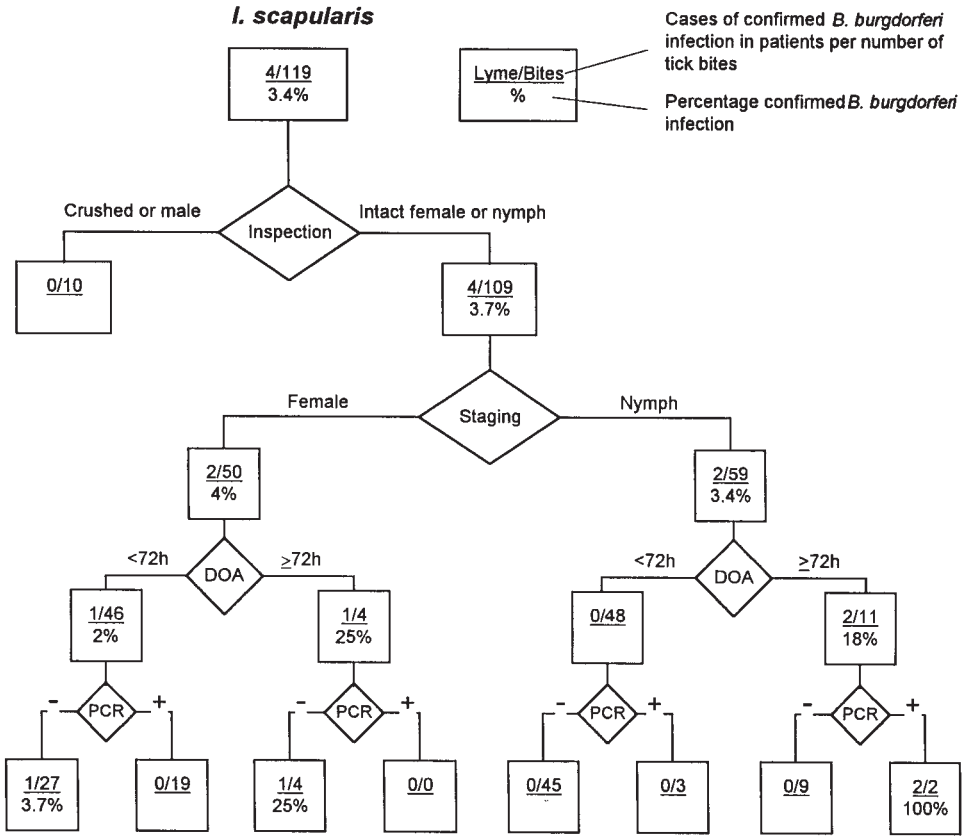

Figure 1. Prevalence of *Borrelia burgdorferi* infection by type of *Ixodes scapularis* bite. Shown only are subjects who completed paired serologic testing. Excludes larvae. DOA = duration of attachment; PCR = polymerase chain reaction.

Discussion

In this study, rigorous criteria for evidence of *B. burgdorferi* infection following a tick bite were used—physician-observed erythema migrans or asymptomatic seroconversion based on meeting criteria for a positive IgG immunoblot [11] at 4–6 weeks, by which time a switch from IgM to IgG antibody occurs in almost all infected persons. The prevalence of *B. burgdorferi* infection was between 1.8% and 3.7%, depending on the population used as the denominator. This is consistent with the 1.2%–3% noted in previous studies in areas in which Lyme disease is endemic [1–3]. However, in these studies the duration of attachment was not assessed.

In the present study, increased duration of attachment beyond 72 h increased the odds of infection considerably, to 18% for a nymphal bite and 25% for a female bite. Whereas most experts would not consider a risk of up to 3.7% as high enough to justify prophylaxis for all persons bitten, an upward revision of the probability of infection to 18%–25% in an individual patient may warrant the consideration of prophylaxis for that patient. An accurate assessment of the duration of attachment would require measurement of the degree of engorgement, as many patients are unable to report an accurate duration.

Therefore, the question posed commonly by the patient: ''Should I have the tick analyzed?'' is a pertinent one. Tick analysis may include any of several tests: identification of the species, stage, and sex, measurement of the scutal index for degree of engorgement, and an assay for presence of *B. burgdorferi* in the tick. On the basis of our results, the probability of infection by the type of tick bite may be used to decide on a course of action. As availability and cost of tick analysis are limiting factors, we need to decide which tests are most helpful. Clearly, examination of the specimen to identify non-ticks and ticks other than deer ticks simplifies the management and reassures the patient. Non–deer tick specimens were 27.5% of submissions in our study. The identification protocol also assigns the stage and sex of the tick. Nymphal and female *I. scapularis* are known to be associated with a higher risk than other *I. scapularis* stages [13]. Measurement of the degree of engorgement allows calculation of the duration of attachment, which identified the subgroups at highest risk for infection in our study, namely nymphal and female *I. scapularis* with duration of attachment ⩾72 h (18% and 25%, respectively). However, a PCR assay for spirochetes in the tick did not accurately predict the occurrence of infection in the person. This was presumably due to inhibition of the reaction in some ticks, as the Taq polymerase used in the assay is subject to inhibition by blood and perhaps other biologic substances in the tick.

On the basis of the findings from this prospective study, we propose tick identification and measurement, without PCR, to identify a reasonable subset of candidates for antibiotic prophylaxis. The high-risk subset consists of persons with nymphal

Table 1. Subjects with *B. burgdorferi* infection.

Subject	Evidence of infection	Serology		*Ixodes scapularis*		
		Baseline	Convalescent	Stage	Duration of attachment (h)	PCR
1	EM	EIA:* 5% IB:† no bands	EIA: 5% IB: no bands	Nymph	118	Positive
2	AS	EIA: 3% IB: 41, 66	EIA: 12% IB: 21, 28, 34, 39, 41, 55, 58, 66	Nymph	84	Positive
3	EM	EIA: 3% IB: 41, 66	EIA: 3% IB: 41,66	Female	14	Negative
4	AS	EIA: 3% IB: 41, 66	EIA: 55% IB: 21, 28, 39, 41, 55, 58, 66, 93	Female Female	85 104	Negative Negative

NOTE. EM, erythema migrans, at site of bite; AS, asymptomatic seroconversion; PCR, polymerase chain reaction.
* Negative <9%.
† Immunoblot (IgG): position of bands (kDa).

or female *I. scapularis* with duration of attachment ⩾72 h, in whom the risk is 18%–25%. If all such subjects in our study population were prescribed antibiotic prophylaxis, it would have resulted in treatment of only 13% (25/193) of deer tick bites in which duration of attachment could be determined, or 8% (25/316) of all specimens submitted as possible deer ticks. This method seems preferable to the common practice of treating all tick bites or treating on the basis of a subjective perception of risk [14], risk estimates derived from arguable mathematical assumptions [4], or the degree of the patient's anxiety [5].

Tick identification and measurement would optimally be done by regional laboratories that currently provide Lyme disease reference testing, but visual assessment of engorgement with the use of a hand lens has also been proposed [15]. It would be useful to study the correlation of visual assessment with the scutal index of engorgement. Moreover, it is conceivable that with minimal training, using preserved specimens, presumptive identification of the species could be done in the practitioner's office.

The efficacy of prophylaxis is uncertain, although if data from the three published tick bite prophylaxis studies are combined [1–3], no subject who received an antibiotic developed Lyme disease, compared with 4 recipients of placebo. As some infections will occur following tick attachment of a short duration, patient observation and education for development of symptoms of Lyme disease after any deer tick bite are necessary.

Acknowledgments

We are grateful to Vincent Bonagura for his generous donation of materials and to Robert Bienkowski for assistance with the depiction of data.

References

1. Costello CM, Steere AC, Pinkerton RE, Feder HM Jr. A prospective study of tick bites in an endemic area for Lyme disease. J Infect Dis **1989**; 159:136–9.
2. Agre F, Schwartz R. The value of early treatment of deer tick bites for the prevention of Lyme disease. Am J Dis Child **1991**;147:945–7.
3. Shapiro ED, Gerber MA, Holabird NB, et al. A controlled trial of antimicrobial prophylaxis for Lyme disease after deer-tick bites. N Engl J Med **1992**;327:1769–73.
4. Magid D, Schwartz B, Craft J, Schwartz JS. Prevention of Lyme disease after tick bites—a cost-effectiveness analysis. N Engl J Med **1992**;327: 534–41.
5. Kassirer JP. Is a tick's bark worse than its bite? Formulating an answer with decision analysis. N Engl J Med **1992**;327:562–3.
6. Piesman J. Dynamics of *Borrelia burgdorferi* transmission by nymphal *Ixodes dammini* ticks. J Infect Dis **1993**;167:1082–5.
7. Piesman J. Dispersal of the Lyme disease spirochete *Borrelia burgdorferi* to salivary glands of feeding nymphal *Ixodes scapularis* (Acari: Ixodidae). J Med Entomol **1995**;32:519–21.
8. Piesman J, Spielman A. Human babesiosis on Nantucket Island: prevalence of *Babesia microti* in ticks. Am J Trop Med Hyg **1980**;29:742–6.
9. Falco RC, Fish D, Piesman J. Duration of tick bites in a Lyme disease–endemic area. Am J Epidemiol **1996**;143:187–92.
10. Sood SK, Zemel L, Ilowite NT. Interpretation of immunoblot in pediatric Lyme arthritis. J Rheumatol **1995**;22:758–61.
11. Centers for Disease Control and Prevention. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. MMWR Morb Mortal Wkly Rep **1995**;44:590–1.
12. Johnson BJB, Happ CM, Mayer LW, Piesman J. Detection of *B. burgdorferi* in ticks by species-specific amplification of the flagellin gene. Am J Trop Med Hyg **1992**;47:730–41.
13. Piesman J. Transmission of Lyme disease spirochetes *(Borrelia burgdorferi).* Exp Appl Acarol **1989**;7:71–80.
14. Meek J, Feder HM Jr, Cartter ML. Tick bite prophylaxis—what Connecticut physicians actually do [abstract P012W]. In: Program and abstracts of the Sixth International Conference on Lyme Borreliosis (Bologna, Italy). Bologna, Italy: Institute of Microbiology, **1994**.
15. Matuschka FR, Spielman A. Risk of infection from and treatment of tick bite. Lancet **1993**;342:529–30.