Determining the Duration of *Ixodes scapularis* (Acari: Ixodidae) Attachment to Tick-Bite Victims

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ABSTRACT The duration of tick attachment is one factor associated with risk for human infection caused by several tick-borne pathogens. We measured tick engorgement indices at known time intervals after tick attachment and used these indices to determine the length of time that ticks were attached to tick-bite victims in selected Rhode Island and Pennsylvania communities where the agents of Lyme disease and human babesiosis occur. The total body length and width as well as the length and width of the scutum were measured on nymphal and adult female *Ixodes scapularis* Say removed from laboratory animals at 0, 12, 24, 36, 48, 60, and 72 h after their attachment. Three engorgement indices were calculated at each time interval. In addition, engorgement indices measurements were recorded for 504 ticks submitted to a commercial laboratory for pathogen detection testing between 1990 and 1992. No detectable change was observed in the average engorgement indices for either nymphal or adult ticks between 0 and 24 h of attachment using any of the engorgement indices. After 24 h of tick attachment, all engorgement indices continuously increased; average indices for nymphs attached 36, 48, and 60 h were significantly different from those attached ≤24 h and from each other. Similarly, average engorgement indices for adult ticks attached ≤36 h were significantly different from those attached for 48 h or more. More than 60% of tick-bite victims removed adult ticks by 36 h of attachment, but only 10% found and removed the smaller nymphal ticks within the first 24 h of tick feeding. The duration of tick attachment may serve as a useful predictor of risk for acquiring various infections, such as Lyme disease and babesiosis, transmitted by *I. scapularis*. Regression equations developed herein correlate tick engorgement indices with duration of feeding. A table containing specific engorgement index prediction intervals calculated for both nymphs and adults will allow the practitioner or clinical laboratory to use easily measured tick engorgement indices to predict transmission risk by determining the duration of feeding by individual ticks.

KEY WORDS *Ixodes scapularis*, Lyme disease, deer tick bites, duration of attachment, engorgement index

THE RISK OF INFECTION with several tick-borne pathogens, including the agents of Lyme disease, human babesiosis, and Rocky Mountain spotted fever, depends directly on the duration of vector tick attachment. For example, before transmission of *Borrelia burgdorferi* can occur, nymphal and adult *Ixodes scapularis* Say usually must be attached to a host for >24 and 36 h, respectively (Piesman et al. 1987, 1991). Sporozoites of *Babesia microti* generally require a minimum of 36–48 h to complete maturation in salivary glands of nymphal *I. scapularis*, and babesial infections in hamsters were transmitted most efficiently after 54 h of tick attachment (Piesman and Spielman 1980). *Rickettsia rickettsii* become "reactivated" during the tick feeding process, and infectious forms are transmitted by *Dermacentor andersoni* Stiles only after a short feeding period (usually 8–10 h) (Spencer and Parker 1924, Hayes and Burgdorfer 1982). These examples of transmission or reactivation delays between tick attachment and transmission may partially explain why the risk of acquiring Lyme disease, and perhaps other tick-borne infections, by people with recognized tick bites is less than might be expected based on the relatively high infection prevalence in ticks (Falco and Fish 1988, Costello et al. 1989, Shapiro et al. 1992, Mather 1993). Ticks can be removed before pathogen transmission, and many probably are.

During tick feeding, the alloscutal length and width of ixodid ticks increases markedly, whereas the dimensions of their hard scutal plate remain unchanged. Tick engorgement indices have been created from ratios that compare scutal and full-body dimensions and have been used in previous studies to describe the time course of tick feeding in relation to parasite development and transmission to animals (Obenchain et al. 1980, Piesman and Spielman 1980). Thus, it may be possible to assess risk for human infection with various tick-

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transmitted agents, especially those with lengthy delays between tick attachment and transmission, by determining a tick engorgement index. To this end, we evaluated various engorgement indices of *I. scapularis* and compared them with the length of time a tick was attached to a host. In particular, we developed regression equations explaining the relationship between the duration of attachment and various engorgement indices for both nymphal and adult female ticks fed on hamsters and rabbits, respectively. We then used these equations to predict the duration of attachment of ticks removed by tick-bite victims in 2 communities in Rhode Island and Pennsylvania.

Materials and Methods

Host-seeking, adult ticks of *I. scapularis* used for experimental infestations were collected from clothing after walking through vegetation (Ginsberg and Ewing 1989) at a heavily tick-infested site located in South Kingstown, Washington County, Rhode Island, during the spring of 1993. All ticks were separated by sex into different vials and stored at 4°C and >95% RH until they were used. Nymphal *I. scapularis* for experimental infestations were derived from larval ticks blood-fed on laboratory-raised white-footed mice (*Peromyscus leucopus*). The larval ticks were from field-derived adult females collected on Prudence Island, RI, during the spring and fall of 1993. Engorged larvae and subsequently derived nymphs were held in vials at 23°C and >95% RH.

To obtain partially engorged adult *I. scapularis*, ticks were placed onto the ears of a New Zealand white rabbit (Charles River, Wilmington, MA) in 2 groups of 50 mating pairs. Ticks were contained on rabbit ears using cloth bags affixed at the ear base with tape. An Elizabethan collar was placed on the rabbit to prevent excessive grooming. After allowing 2–3 h for tick attachment, the ear bags were opened and all nonattached ticks were removed. At time intervals of 12, 24, 36, 48, 60, and 72 h after ticks were attached, 10 ticks were removed from the rabbit's ear by traction using fine-pointed forceps, taking care not to damage the mouth of the tick. Partially engorged nymphal *I. scapularis* were obtained in a similar way, except they were attached to 3 Syrian golden hamsters (Charles River) held in small, wire mesh restraining cages and wrapped in paper. Totals of 100 nymphs were placed on hamsters; after 2-3 h, all nonattached ticks were removed, and animals were placed singly into larger cages with wire mesh bottoms held over pans of water. At time intervals of 12, 24, 36, 48, and 60 h following their attachment, 30 nymphs were removed (10 nymphs per time interval from each hamster). Research involving laboratory animals was approved by the University of Rhode Island Animal Care and Use Committee, and approved protocol #A9394-026 is on file in the laboratory of T.N.M.

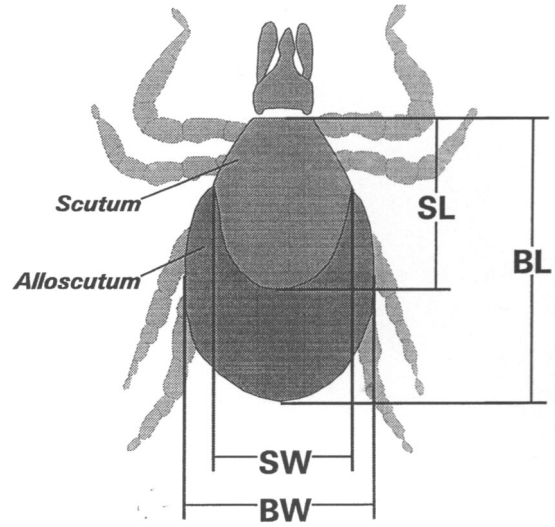

Fig. 1. Diagram illustrating external body parts of *I. scapularis* used for measuring engorgement indices 1–3 (SL, scutal length; BL, body length; SW, scutal width; BW, alloscutal (body) width).

All ticks removed from hosts were anesthetized by transferring them to a 2-ml vial containing cotton and a drop of halothane (Halocarbon, North Augusta, SC). Ticks were weighed, then moved onto a glass microscope slide and covered lightly with a glass coverslip to flatten the body (as much as the weight of the coverslip would do). Tick body measurements were made with the aid of a micrometer held in the eye piece of a dissecting stereo microscope. The 4 areas of measurements included (1) total body length, defined as the distance on the midline between the anterior edge of the scutum and the posterior tip of the opisthosoma; (2) scutal length, defined as the midline distance between the anterior edge and the posterior tip of the scutum; (3) maximum scutal width, measured at the widest point on the scutum; and (4) maximum alloscutal width, measured at the widest point on the alloscutum (see Fig. 1).

Engorgement indices were computed as the ratios between total body length and scutum length (index 1), total body length and scutum width (index 2), and alloscutum width and scutum width (index 3). One-way analysis of variance (ANOVA) was performed to compare the 3 scutal indices of preattached adult and nymphal *I. scapularis* with those of ticks removed from hosts at each 12-h time interval (SAS Institute 1988). Duncan multiple range tests were used to compare mean engorgement indices for each time interval. Stepwise regression analysis with true stepping (to enter = 0.15, to remove = 0.20) was used to evaluate relationships between the known duration of tick attachment and each engorgement index. Seventy-five percent prediction intervals (for predicting outcomes from individual tick engorgement indi-

ces) were calculated (Runyon 1985, SAS Institute 1988).

To predict the duration of attachment of ticks removed by tick-bite victims, the total body length and scutum length (Index 1) was obtained from 128 adult female (101 from Rhode Island, 27 from Pennsylvania) and 377 nymphal *I. scapularis* (206 from Rhode Island and 170 from Pennsylvania), submitted to a commercial laboratory for detection of *B. burgdorferi* (CBR, Boston, MA). For our analysis, we included measurements of only those ticks sent from patients residing in either Washington County, Rhode Island, or Bryn Athyn, Montgomery County, Pennsylvania, because these 2 localities represented the largest sources of ticks submitted to the laboratory. Duration of tick attachment was determined using the regression of index 1 on length of tick attachment to animals in the laboratory.

Results

Both the total body length and alloscutal width of nymphal and adult ticks increased with duration of feeding but their corresponding scutal length and width remained relatively constant (Fig. 2). Actually, scutal length measurements decreased during the 72 h observation period (nymphs, $F = 101.01$; df = 5, 174; $P < 0.05$ and adults, $F = 5.64$; df = 6, 63; $P < 0.05$), but we believe this decrease was only in the appearance of scutal length and could be attributed to scutum tilting as the alloscutum became engorged. The angle at which the engorged tick scutum tilted was calculated using the $\cos^{-1}$ of the ratio between the measured (apparent) scutal length and the mean scutal length of 30 unfed nymphs or adults. Thus, the mean angle of tilt for nymphal ticks was 16.9 ± 7.8°, 24.0 ± 4.9°, and 39.3 ± 5.5° at 36, 48, and 60 h of attachment, respectively, and the angle of tilt for adult ticks at 72 h of attachment was 24.5 ± 6.2°. Scutal widths of both nymphal and adult ticks remained unchanged throughout the entire period of observation (nymphs, $F = 0.438$; df = 5, 174; $P > 0.05$ and adults, $F = 1.82$; df = 6, 63; $P > 0.05$).

Engorgement indices of both nymphal and adult *I. scapularis* increased with increasing duration of attachment to hamsters and rabbits. Significant overall differences were noted for both adult female (index 1: $F = 29.01$; df = 6, 63; $P < 0.05$; index 2: $F = 23.74$; df = 6, 63; $P < 0.05$; index 3: $F = 31.76$; df = 6, 63; $P < 0.05$) and nymphal ticks (index 1: $F = 216.33$; df = 5, 174; $P < 0.05$; index 2: $F = 173.09$; df = 5, 174; $P < 0.05$; index 3: $F = 160.08$; df = 5, 174, $P < 0.05$) when engorgement indices at various time intervals of tick attachment were compared. No differences were observed in any of the nymphal or adult engorgement indices for ticks attached ≤24 h. However, mean engorgement indices of nymphal ticks attached ≤24 h were significantly different from those attached ≥36, 48, and 60 h of attachment. By 72 h of attachment, 78.8% of nymphal ticks were fully engorged and had actually detached from their host. For adult female ticks, mean engorgement index values calculated using index 1 or 3 for 0, 12, and 24 h of attachment were significantly different from EIs determined at 36, 48, 60, and 72 h of attachment. Using Index 2, we were unable to distinguish between adult ticks attached for ≤24 h and those attached for 36 h, although ticks attached for ≥48 h could be distinguished from those attached for a shorter duration.

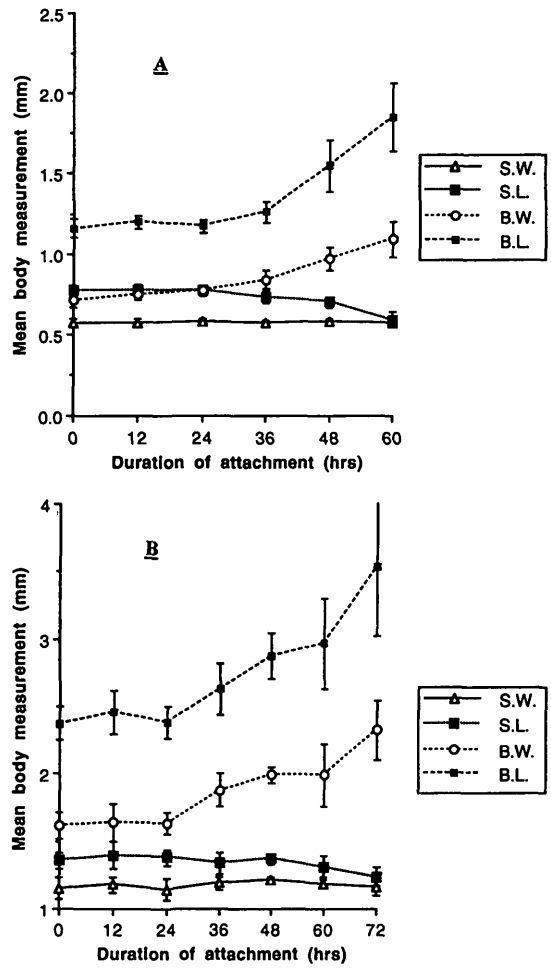

Fig. 2. Comparison of mean ± SD external body measurements of *I. scapularis* nymphs (A) and adults (B) attached to laboratory hosts for varying lengths of time.

We used stepwise multiple regression to determine relationships between duration of tick attachment and engorgement indices. Time of attachment explained a substantial amount of variation in each engorgement index. However, the derived equations differed for each engorgement index and life stage (nymphal ticks: index 1 = $1.37 * 10^{-7} * h$ [of attachment]^4 + 1.52; $R^2 = 0.86$; $F = 1081.22$; df = 1, 178; $P < 0.001$; index 2 = $5.7 * 10^{-6} * h^3$ + 2.01; $R2 = 0.83$; $F = 858.94$; df = 1, 178; $P <$

Table 1. Seventy-five percent prediction intervals of nymphal and adult *I. scapularis* engorgement indices for predicting duration of tick attachment

Tick stage	Duration of attachment, h	Engorgement indices[a]		
		Index 1	Index 2	Index 3
Nymph	0–24	1.46–1.62	1.91–2.18	1.18–1.43
	36	1.57–1.89	2.12–2.44	1.36–1.62
	48	1.88–2.50	2.32–2.96	1.54–1.82
	60	2.56–3.74	2.83–3.67	1.69–2.15
Adult	0–24	1.67–1.87	1.98–2.25	1.30–1.53
	36	1.72–2.04	1.98–2.34	1.42–1.64
	48	1.93–2.21	2.12–2.46	1.58–1.70
	60	2.01–2.73	2.20–2.92	1.64–2.02
	72	2.17–3.49	2.47–3.63	1.69–2.21

[a] Index 1, body length/scutal length; index 2, body length/scutal width; index 3, alloscutal width/scutal width.

0.001; index 3 = 1.8 $*10^{-4}$ $*h^2$ + 1.25; R^2 = 0.82; F = 816.80, df = 1, 178; $P < 0.001$; adult ticks: index 1 = 2.9 $*10^{-6}$ $*h^3$ + 1.74; R^2 = 0.73; F = 179.23; df = 1, 68; $P < 0.001$; index 2 = 3.5 $*10^{-8}$ $*h^4$ + 2.10; R^2 = 0.68; F = 144.49; df = 1, 68; $P < 0.001$; index 3 = 1.1 $*10^{-4}$ $*h^2$ + 1.39; R^2 = 0.71; F = 164.29; df = 1, 68; $P < 0.001$). For each 12-h period, we estimated the 75% prediction interval for all engorgement indices. Because we observed no significant difference in EIs for ticks attached ≤24 h, we combined prediction intervals for 0, 12, and 24 h (Table 1).

In addition to increasing engorgement indices with duration of attachment, both nymphal and adult ticks increased in body weight, and adult ticks changed colors as they blood-fed. The average body weights for 30 nymphs at 0, 12, 24, 36, 48, and 60 h of attachment were 0.13, 0.20, 0.23, 0.33, 0.53, and 0.77 mg, respectively. Average body weight for 30 adult ticks attached for ≤24 h was 1.5 mg, and body weights (n = 10) gradually increased to 2.7, 3.5, 3.8, and 6.5 mg at 36, 48, 60, and 72 h of attachment, respectively. In female *I. scapularis*, the alloscutum was red (10R 4/6-4/8-Munsell color charts, Kollmorgen, Newburgh, NY) in unfed ticks and in ticks feeding for 12 or 24 h. The alloscutum became yellowish red (5YR 5/6–4/6) after 36 and 48 h of attachment. After 60–72 h, the color of these ticks changed to light brown (7.5YR 6/4–5/4). It was difficult to measure the engorgement of fully engorged ticks, but following detachment from the host, fully engorged adult females were greenish gray (5GY 5/1) to dark greenish gray (5GY 4/1). It took at least 5 d for adult female *I. scapularis* to become fully engorged, at which time their mean body weight was 261 mg.

Using the tick stage-specific regression equations for engorgement index I, we determined the duration of attachment for nymphal and adult ticks removed by tick-bite victims from communities in Rhode Island and Pennsylvania. Most people (64%, n = 128) found and removed adult *I. scapularis* before 36 h of attachment, and by 48 h nearly all adult ticks were removed (79%) (Fig. 3).

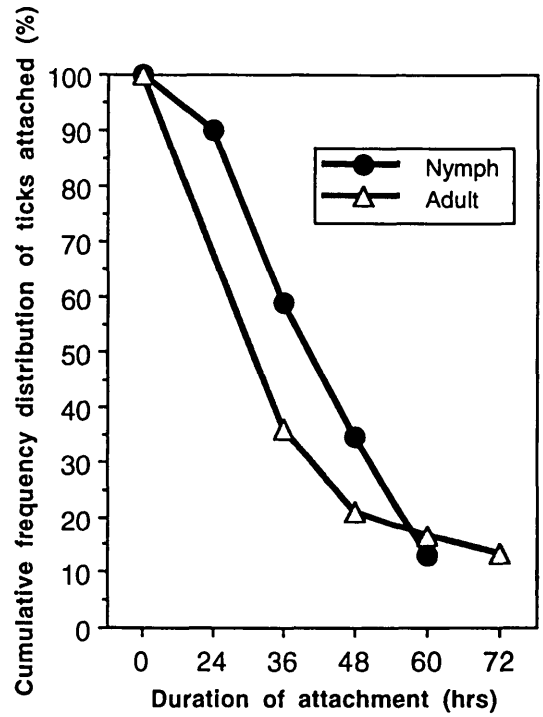

Fig. 3. Cumulative frequency distribution (%) showing the duration of nymphal and adult *I. scapularis* attachment to human hosts in Rhode Island and Pennsylvania.

In contrast, few people found and removed nymphal ticks by 24 h of attachment (10%, n = 377), although more nymphs were removed by 36 h (41%).

Discussion

Several reports have been published showing that the risk of contracting Lyme disease following a tick bite is exceptionally low despite relatively high rates of *B. burgdorferi* infection in tick populations. Prospective studies of human infection following a tick-bite from Connecticut, New York, and West Germany indicate a frequency of human infection with *B. burgdorferi* ranging from 1 to 4% (Paul et al. 1986, Falco and Fish 1988, Costello et al. 1989, Nadelman et al. 1992, Shapiro et al. 1992) whereas the infection prevalence in ticks, at least in the northeastern United States, generally ranges from 20 to 35% for nymphs and 50 to 75% for adults (Mather 1993). Duration of tick attachment is commonly cited as an important factor determining the probability of infection, although studies have yet to be conducted that relate the length of time that a tick feeds to the incidence of Lyme disease or any other tick-borne infection.

Lack of a convenient, reliable, and objective measure for determining the duration of tick attachment is often cited by clinicians as a principal limitation to assessing risk of infection following a

tick bite (Magid et al. 1992, Shapiro et al. 1992). The notion of using engorgement indices to measure duration of tick feeding is not new (Obenchain et al. 1980). However, the missing parameter, for infections transmitted by *I. scapularis* (and for most other vector ticks), has been in comparing length of tick attachment with a particular index of engorgement. In this experiment, we made 4 simple measurements on ticks (Fig. 1) assisted only by a dissecting microscope and ocular micrometer, developed regression equations for 3 separate engorgement indices at relevant time intervals for both nymphal and adult ticks, then generated a table presenting the 75% prediction intervals (Table 1). For the practitioner, only 1 engorgement index need be calculated. There was no real advantage of 1 engorgement index over any other in their ability to discern differences in length of tick attachment. In practice, we found index 1 (total body length/scutal length) to be the simplest and most accurate engorgement index to measure because it required no additional manipulation of the tick once it was on the microscope stage. Furthermore, both measurements for index 1 are taken along the tick's midline, making a subjective judgment as to the scutum's widest axis unnecessary. However, we associated all 3 measures with their respective attachment times to provide a means of making at least 1 accurate measurement in the event that a particular patient's tick was damaged. Commonly, after someone removes a tick, the proximal part of the scutal plate will be missing, making accurate measurement of Indices 1 and 2 difficult.

In practice, it is important to compare the engorgement index of an individual tick to the prediction interval rather than just calculating the duration of attachment from the regression equation. Determining tick attachment time from the regression line alone affords no idea of a given prediction's accuracy for individual ticks removed from patients because it fails to consider errors inherent in fitting the line, or in the population variance. Moreover, confidence intervals that would typically be calculated by standard statistical software packages are useful only in describing the accuracy of the fitted regression line for a population and are inappropriate for predicting the duration of attachment by an individual tick. The prediction intervals calculated herein (Table 1) include consideration of the population variance for tick engorgement around the best-fit regression line.

Animal studies suggest that risk for *B. burgdorferi* transmission is low within the first 24 h of attachment but increases thereafter. Rodents infested with *B. burgdorferi*-infected nymphal *I. scapularis* for 36 h showed a 7% risk of infection, those attached for 48 h >50% risk, whereas those infested for 72 h were universally infected (Piesman et al. 1987, Piesman 1993). Similarly, adult *I. scapularis* feeding for ≤36 h failed to infect rabbits, whereas 2 of 3 rabbits became infected when ticks were attached for 48 h, and 5 of 5 rabbits were infected when attachment was >120 h (Piesman et al. 1991). Variability in tick feeding rates in relation to spirochete transmission among different tick hosts has not been determined, making it impossible to know if findings from these animal studies are applicable to humans. Similarly designed studies using a wider array of animal hosts may provide some evidence toward the degree of interspecies variation that exists in tick feeding rates.

We propose that the true likelihood of becoming infected can be predicted by (1) the duration of tick attachment, (2) the tick infection rate, and (3) the efficiency of transmission at a particular stage of tick attachment. In our study, 10% of attached nymphs were removed in ≤24 h, 41% in ≤36 h, 65% in ≤48 h, and 87% in ≤60 h. Likewise, 64% of adult ticks were removed in ≤36 h, 79% in ≤48 h, 84% in ≤60 h, and 87% in ≤72 h. Assuming nymphal tick infection rates between 20 and 35% and transmission efficiencies of 7, 50, and 100% at 36 h, 48 h, and >60 h, respectively, the probability of infection following a nymphal tick bite would likely range from 0.10 where *B. burgdorferi* prevalence in ticks is low to 0.17 where prevalence in ticks is high. Similarly, assuming adult tick infection rates between 50 and 75% and transmission efficiencies of 0, 66, and 100% at 36 h, 48–72 h, and >72 h, respectively, risk for human infection following an adult tick bite would likely range from 0.14, where prevalence in ticks is low, to 0.21, where prevalence is high. The infection probabilities calculated herein are slightly higher than those observed in the few previous clinical studies available, but tick infection rates or the study participants' tick-finding habits may have differed somewhat from those generalizable to the Rhode Island and Pennsylvania communities reported here. Moreover, we used transmission efficiencies from animal studies in making our calculations, which may not be quite the same for humans.

The model described above suggests that infection risk is about the same for people bitten by either nymphal or adult ticks. However, numerous epidemiological studies suggest that many more cases of Lyme disease are acquired in the summer when nymphal ticks are most abundant (Piesman et al. 1987, Fish 1993). It may be that human exposure to nymphs is greater, making nymph bites more common because this life stage can be more abundant than adult ticks by an order of magnitude (Fish 1993). Moreover, peak abundance of nymphs during the summer coincides with higher levels of human activity outdoors than during the late fall and early spring when adult *I. scapularis* are most abundant (Piesman et al. 1987). Because our findings suggest that the risk of infection following tick bite is similar, it is likely that people are bitten more frequently by nymphs than by adults.

Prophylactic antibiotic treatment of tick bites continues to be controversial, particularly in areas where Lyme disease occurs. Proponents of prophylactic therapy argue that even though *B. burgdorferi* and *B. microti* are transmitted mainly in the latter stages of tick feeding, earlier transmission of a small number of organisms has not been ruled out (Liegner 1990). Moreover, decision analysis has suggested that empirical treatment ("treat all") be recommended when the probability of infection is above 0.01 (Magid et al. 1992). In contrast, opponents of prophylactic treatment cite the apparent low probability of infection even after tick bite and risk of adverse drug reactions in defending their position. In addition, inappropriate tetracycline treatment of a tick-bite leading to Rocky Mountain spotted fever could prove problematic because such treatment may postpone the onset of symptoms, perhaps leading to a delay in diagnosis and proper treatment of this serious tick-transmitted infection (Weber and Walker 1991). In this study we developed a simple and objective means for determining the duration of tick attachment. Because attachment of nymphs for <36 h and adult ticks for <48 h pose relatively little risk, at least for transmission of Lyme disease spirochetes, prophylactic therapy reasonably could be reserved for those patients presenting with a deer tick whose engorgement index suggests a longer period of attachment. We recommend additional testing to detect infection in the tick, which would provide an even better estimate of infection probability.

Acknowledgments

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