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NOTES

Duration of Tick Attachment and *Borrelia burgdorferi* Transmission

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Nymphal *Ixodes dammini* transmitted *Borrelia burgdorferi* to 1 of 14 rodents exposed for 24 h, 5 of 14 rodents exposed for 48 h, and 13 of 14 rodents exposed for ≥72 h. Prompt removal of attached ticks is a prudent public health measure, especially in regions where Lyme disease is endemic.

The etiologic agent of Lyme disease is a tick-borne spirochete, *Borrelia burgdorferi* (5). The principal vectors transmitting *B. burgdorferi* to residents of the northeastern United States are nymphal *Ixodes dammini* (3, 8, 12, 14, 15). Prompt treatment with antibiotics may ameliorate the arthritic, neurological, and cardiac sequelae of infection with *B. burgdorferi*; however, treatment may be delayed, especially in patients not expressing the characteristic rash erythema chronicum migrans (13, 16). Consequently, avoidance of ticks in the endemic region is the principal preventive measure available to limit the rapidly increasing incidence of Lyme disease. When avoidance of ticks is impossible, residents of endemic regions are often advised by local public health authorities and physicians to remove ticks as quickly as possible. Presumably, prompt removal of ticks decreases risk of *B. burgdorferi* transmission. However, experimental evidence demonstrating the association between duration of tick feeding and efficacy of spirochete transmission is presently lacking. Therefore, we exposed hosts to *B. burgdorferi*-infected nymphal *I. dammini* for measured time intervals in order to determine the relationship between duration of tick feeding and spirochete transmission.

Golden Syrian female hamsters (50 to 150 g; Charles River Breeding Laboratories, Inc., Wilmington, Mass.) and white-footed mice (*Peromyscus leucopus*) were used as hosts in this study. Hamsters are readily infected by *B. burgdorferi* (4); *P. leucopus* is a natural host for immature *I. dammini* and serves as an important reservoir of *B. burgdorferi* (1, 7, 10). White-footed mice were obtained from a 4-year-old laboratory colony derived from individuals collected in eastern Massachusetts. All ticks used in this study were derived from adult *I. dammini* collected from vegetation on Great Island, Yarmouth, Mass.

The strain of *B. burgdorferi* used in these experiments (strain JD1) originated from naturally infected nymphal *I. dammini* collected from vegetation at Crane's Beach, Ipswich, Mass. A group of 20 to 30 field-collected nymphs were allowed to feed on a colony-bred *P. leucopus*. The infected mouse then served as a host to laboratory-reared larval *I. dammini*. These larvae were allowed to molt to nymphs and served as the sources of *B. burgdorferi* used in these experiments. Strain JD1 spirochetes, in five nymphal *I. dammini*, exhibited bright fluorescence when they were

reacted in an indirect fluorescent antibody test with monoclonal antibody H5332, which is specific for *B. burgdorferi* (2). This reaction supported the identification of strain JD1 spirochetes as *B. burgdorferi*.

We used a xenodiagnostic procedure to detect *B. burgdorferi* in hosts. Laboratory-reared xenodiagnostic larval *I. dammini* (F_1 or F_2 generation in the laboratory), from individual egg clusters, were divided into two groups. One group from each egg cluster fed on test rodents; the other group from each egg cluster fed on noninfected hamsters. All ticks were allowed to molt to nymphs and were examined for spirochetes as described below. None of the 217 *I. dammini* larvae (derived from 18 egg clusters) which fed on noninfected control hamsters contained spirochetes when they were examined as nymphs. Our *I. dammini* colony proved to be free of spirochetes. Thus, spirochetes observed in xenodiagnostic larvae fed on test rodents were presumably acquired from those hosts. Serological or cultural methods for detecting spirochetes in hosts were not utilized in this study.

A total of 10 to 20 xenodiagnostic ticks from each test rodent were dissected and examined for spirochetes by using dark-field microscopy, as well as a direct fluorescent antibody probe, as previously described (9).

Nymphal *I. dammini* from groups of highly infective ticks (90 to 100% infected) were used to infect test hosts. Three nymphs were placed onto each experimental rodent and the rodents were held in wire restraining cages placed over water. Ticks not attaching to hosts were replaced on hosts during 0.5 h postexposure. At 24 or 48 h after tick attachment, the rodents were anesthetized with sodium pentobarbital and carefully examined for ticks. All attached ticks were removed and examined for spirochetes. In an additional group, ticks were allowed to feed to repletion. All nymphs in this group (designated ≥72 h) dropped off hosts during day 4 postattachment. Rodents exposed to ticks for 24, 48 or ≥72 h were held over water for at least 4 days to be certain that feeding ticks were not overlooked. Rodents from which ticks were overlooked and fed for more than the designated time interval were discarded from the experimental analysis. Similarly, hosts were disregarded if none of the ticks fed for the designated time interval or if only spirochete-negative ticks were found. A total of 1 to 3 spirochete-infected nymphs fed on each test animal. At 3 to 4 weeks after infective feeding, exposed rodents served as hosts for xenodiagnostic larval *I. dammini*. After repletion, xeno-

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TABLE 1. Duration of nymphal *I. dammini* attachment to rodents and efficacy of *B. burgdorferi* transmission

Duration of attachment of infected nymphs (h)	Hamsters		White-footed mice	
	No. of infected nymphs attached	Proportion of xenodiagnostic ticks infected	No. of infected nymphs attached	Proportion of xenodiagnostic ticks infected
24	1	0/10	1	0/20
	2	0/10	2	0/20
	2	9/10	2	0/20
	3	0/10	2	0/10
	3	0/10	3	0/20
	3	0/10	3	0/20
			3	0/20
			3	0/10
48	1	0/10	1	0/20
	2	0/10	1	0/20
	2	10/10	2	0/20
	2	0/10	2	0/10
	3	9/10	2	19/20
	3	6/10	2	0/20
			3	0/20
			3	8/10
≥72	1	7/10	1	20/20
	2	10/10	NR[a]	19/20
	2	8/10	2	15/20
	2	7/10	2	10/10
	3	0/10	2	19/20
	3	10/10	2	17/20
			3	19/20
			3	10/10

[a] NR, Not recorded.

diagnostic larvae were held at 21°C, allowed to molt to nymphs, and examined for spirochetes.

The duration of tick attachment was closely associated with the efficacy of spirochete transmission to hamsters and white-footed mice (Table 1). When ticks fed for 24 h, only one of six hamsters and none of eight mice became infected. In contrast, ticks attached for 48 h transmitted infection to three of six hamsters and two of eight mice. Finally, ticks feeding to repletion (≥72 h) transmitted infection to five of six hamsters and all eight mice tested. The proportion of hosts infected was significantly correlated with duration of tick attachment to hamsters ($r = 1.000$; $P < 0.01$) and mice ($r = 0.961$; $P < 0.01$). Based on the regression line, the time required until 50% of the hosts became infected was 48.0 h in hamsters and 52.1 h in mice.

Our study supports the conclusion that prompt removal of *I. dammini* diminishes risk of infection with *B. burgdorferi*. We were not able to establish a minimum time of tick feeding below which transmission did not occur. In fact, one hamster exposed for the minimum time interval tested, 24 h, did become infected. However, since risk of transmission increased with duration of tick attachment, prompt tick removal remains a prudent disease prevention measure and should be recommended to residents of endemic regions.

Transmission risk of an additional pathogen transmitted by nymphal *I. dammini*, *Babesia microti*, is similarly affected by duration of tick attachment (11). *Babesia microti* sporozoites undergo a cycle of maturation and multiplication in tick salivary glands during feeding; this phenomenon explains the relationship between duration of tick feeding and *Babesia microti* transmission (6). The actual mechanism of *B. burgdorferi* transmission to hosts is poorly understood.

Regurgitation, saliva, and feces are all possible routes for *B. burgdorferi* transmission. Our understanding of how duration of tick feeding interacts with *B. burgdorferi* transmission efficacy will be enhanced when the mechanism of tick transmission of these spirochetes is defined.

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