JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1993, p. 2878–2881
0095-1137/93/112878-04$02.00/0

Vol. 31, No. 11

# Accelerated Transmission of Lyme Disease Spirochetes by Partially Fed Vector Ticks

CHIEN-MING SHIH† AND ANDREW SPIELMAN*

*Department of Tropical Public Health, Harvard School of Public Health, 665 Huntington Avenue, Boston, Massachusetts 02115*

Received 26 May 1993/Returned for modification 13 July 1993/Accepted 17 August 1993

**To determine how rapidly Lyme disease spirochetes (*Borrelia burgdorferi*) can be transmitted by partially fed vector ticks (*Ixodes dammini*), attached nymphs were removed from their hosts at various intervals postattachment and subsequently permitted to refeed to repletion on noninfected mice. We confirm previous reports that ticks deposit Lyme disease spirochetes in the skin of their hosts mainly after 2 days of attachment. Those that have been removed from a host within this interval can reattach and commence feeding. Spirochete-infected nymphs that have previously been attached to a host for 1 day become infectious to other hosts within another day. Noninfected nymphs acquire infection from spirochete-infected hosts within a day of attachment and become infectious to other hosts 3 to 5 days later. Virtually all ticks transmitted infection when reattaching after first feeding for 2 days. We conclude that partially fed nymphal ticks transmit spirochetal infection more rapidly than do ticks that have never been attached to a host and that infected ticks become infectious before they molt.**

The duration of attachment of a vector tick infected by the Lyme disease spirochete (*Borrelia burgdorferi*) profoundly affects the likelihood that this pathogen will enter the animal on which the tick is feeding (14, 16). Transmission occurs mainly after the infecting tick has been attached to its host for 2 days. *Ixodes* ticks, however, frequently become detached from their hosts because of grooming or certain other host-associated factors (4, 5, 25, 26) that limit their ability to feed. The consequences of such interrupted feeding remain to be defined.

The chain of events that culminates in migration of the spirochetes from the gut of the tick to its salivary apparatus begins within the first day of attachment and requires at least another day for completion (27). It may be, however, that a brief period of prior feeding by a spirochete-infected tick potentiates transmission during a subsequent episode of attachment. In addition, noninfected ticks feeding on a spirochete-infected host may similarly be capable of rapid transmission in the event that spirochetes disseminate soon after they are ingested. A tick may become infected after feeding only for about 8 h (12, 21) and might become infectious to another host soon thereafter if it reattaches. For these reasons, the critical period required for partially fed nymphal deer ticks (*Ixodes dammini*) to transmit the agent of Lyme disease may be less than that required by a nonfed tick.

It may be that spirochetal infection is more rapidly transmitted by partially fed deer ticks than by those that have not previously been in contact with a host. To explore this possibility, we determined when Lyme disease spirochetes were transmitted by nymphal deer ticks that had been attached to hosts for various periods of time. In particular, we defined the critical infectious period of subsequent attachment of a spirochete-infected tick feeding on a noninfected host as well as that of a noninfected tick feeding on a spirochete-infected host.

## MATERIALS AND METHODS

**Ticks.** The ticks used in this study were the second-generation progeny of adult deer ticks originally swept from the Crane Wildlife Reservation (Ipswich, Mass.). This colony has been reared and maintained free of inherited spirochetal infection, as described previously (20). The spirochete-infected nymphs used in this study were derived from engorged larvae that had fed on laboratory mice that had previously been bitten by three to five infected nymphs. All ticks were stored in mesh-covered, plaster-bottom plastic vials at 22°C, 95% relative humidity, and 16 h of light per day.

**Animals.** Groups of 3- to 4-week-old CD-1 mice (Charles River Laboratories, Wilmington, Mass.) were used in these experiments. Each group contained six mice, and experiments were replicated once. All mice were caged at an ambient temperature of 22°C and 16 h of light per day.

**Spirochete strain.** The spirochetal isolate (JD1) used in this study was originally derived from naturally infected deer ticks swept from vegetation at the site at which the tick colony originated (15). This isolate reacts with monoclonal antibody H5332 (2) and *B. burgdorferi*-specific DNA probes (18). It is highly infectious to rodent hosts (7, 11, 13, 14, 20) and is maintained in a system of alternate passage between deer ticks and laboratory mice (20).

**Attachment of ticks.** To ensure successful attachment, groups of spirochete-infected and noninfected mice were anesthetized by injection with 0.2 ml of pentobarbital solution (0.1%) and generally shaved with an animal clipper. Infected and noninfected nymphal deer ticks were randomly placed on noninfected and infected mice, respectively. To infect nymphal deer ticks, 25 to 30 noninfected ticks were permitted to feed on spirochete-infected mice 2 months after the mice were exposed to bites from infected nymphs. The entire process of attachment required by ticks may vary from a few minutes to an hour. Thus, the duration of tick

* Corresponding author.
† Present address: Department of Parasitology and Tropical Medicine, National Defense Medical Center, Taipei, Taiwan, Republic of China.

TABLE 1. Duration of attachment of spirochete-infected nymphal deer ticks (*I. dammini*) and transmission of Lyme disease spirochetes (*B. burgdorferi*)

| Duration of attachment (h) and trial no. | Infection in mice | | |
|---|---|---|---|
| | No. tested | % Infected | % Seropositive |
| 16 | | | |
| 1 | 2 | 0 | 0 |
| 2 | 4 | 0 | 0 |
| 3 | 2 | 0 | 0 |
| Total | 8 | 0 | 0 |
| 24 | | | |
| 1 | 2 | 0 | 0 |
| 2 | 4 | 0 | 0 |
| 3 | 3 | 0 | 0 |
| Total | 9 | 0 | 0 |
| 36 | | | |
| 1 | 2 | 0 | 0 |
| 2 | 3 | 0 | 0 |
| 3 | 2 | 50 | 50 |
| Total | 7 | 14 | 14 |
| 48 | | | |
| 1 | 2 | 100 | 100 |
| 2 | 4 | 100 | 100 |
| 3 | 4 | 100 | 100 |
| Total | 10 | 100 | 100 |
| 72 | | | |
| 1 | 1 | 100 | 100 |
| 2 | 3 | 100 | 100 |
| 3 | 2 | 100 | 100 |
| Total | 6 | 100 | 100 |

attachment was measured from 1 h after ticks were placed on their hosts. Those ticks that failed to attach to hosts within 1 h were removed and discarded. Attached nymphal deer ticks were removed from their hosts at various intervals by gentle extraction with forceps. Nymphs were subsequently stored in separate mesh-covered vials for 3 to 5 days, and any tick with disrupted mouthparts was discarded.

**Detection of infection.** A xenodiagnostic procedure was used to determine whether mice sustained persistent infection. Thus, at least 50 laboratory-reared noninfected larval deer ticks were permitted to feed on each mouse 4 weeks after it was bitten by an infecting nymphal deer tick. After xenodiagnosis, 10 engorged larvae and 5 to 10 derived nymphs were dissected and examined for the presence of spirochetes by direct immunofluorescent-antibody assay and dark-field microscopy, respectively. Serological evidence of infection was confirmed by seroconversion at 1:100 of antibody against *B. burgdorferi* antigen (20).

## RESULTS

In a confirmatory experiment, we determined when an infected nymphal deer tick deposits a spirochetal inoculum in a mammalian host. Thus, spirochete-infected nymphal ticks which fed on noninfected mice were removed at intervals thereafter. No spirochetal infection was detected in mice when the attached ticks were removed in less than 36 h, and all mice became infected when the period of attachment was longer (Table 1). Ticks deposit Lyme disease spirochetes in the skin of their hosts mainly after 2 days of attachment.

TABLE 2. Ability of partially fed nymphal deer ticks to reattach after feeding on hosts for various periods of time

| Duration of attachment (h) | Reattachment of nymphal ticks | |
|---|---|---|
| | No. tested | % Reattaching |
| 16 | 18 | 90 |
| 24 | 22 | 82 |
| 36 | 20 | 70 |
| 48 | 16 | 63 |
| 72 | 15 | 0 |
| Total | 91 | 64 |

We assessed the ability of a nymphal deer tick to reattach to a host after a period of interrupted feeding. Thus, attached nymphal deer ticks were removed from their hosts at various intervals after attachment and permitted to reattach to other hosts. Virtually all nymphal ticks that previously had fed for 16 h reattached efficiently (Table 2). Reattachment was less efficient after these nymphs fed for 48 h, and no ticks reattached when they fed for more than 72 h. Ticks that have been removed from a host within 2 days of attachment can reattach and commence feeding.

We then determined how rapidly infected nymphal ticks transmit Lyme disease spirochetes when they have reattached after a period of previous nymphal feeding. Thus, spirochete-infected nymphal ticks were removed from hosts at various intervals following initial attachment and three to five removed nymphs were permitted to refeed on each noninfected host 3 to 5 days later. Spirochetal infection was detected in all but one mouse, on which refed ticks had fed for 24 h, and all mice became infected when exposed to ticks that had refed for 48 h (Table 3). We conclude that spirochete-infected nymphs that have previously been attached to a host for 1 day become infectious to other hosts within 1 day.

Finally, we determined whether ticks that acquire spirochetal infection during the nymphal instar can transmit infection to another host within the same instar. Nymphal deer ticks that had fed on spirochete-infected mice for various periods of time were removed and permitted to reattach to noninfected mice until fully engorged. Nymphal ticks generally acquired infection within 16 h of attachment to an infected host (Table 4). Such infected ticks invariably infected other hosts when permitted to reattach 3 to 5 days later. Noninfected nymphs acquire infection from spiro-

TABLE 3. Transmission of Lyme disease spirochetes by infected nymphal deer ticks that had reattached after feeding for various periods of time on noninfected mice

| Duration of feeding (h) | | Infection in mice | |
|---|---|---|---|
| Initial | After reattachment | No. tested | % Infected |
| 24 | 24 | 6 | 83 |
| | 48 | 6 | 100 |
| 36 | 24 | 6 | 83 |
| | 48 | 6 | 100 |
| 48 | 24 | 6 | 100 |
| | 48 | 6 | 100 |

TABLE 4. Transmission of Lyme disease spirochetes by previously noninfected nymphal deer ticks that were detached while feeding on infected mice and subsequently permitted to engorge on noninfected mice

| Duration of previous attachment (h) and trial no. | Infection in mice | |
|---|---|---|
| | No. tested | % Infected |
| 16 | | |
| 1 | 2 | 50 |
| 2 | 3 | 100 |
| 3 | 2 | 100 |
| Total | 7 | 86 |
| 24 | | |
| 1 | 1 | 100 |
| 2 | 2 | 100 |
| 3 | 2 | 100 |
| Total | 5 | 100 |
| 36 | | |
| 1 | 2 | 100 |
| 2 | 1 | 100 |
| 3 | 3 | 100 |
| Total | 6 | 100 |
| 48 | | |
| 1 | 2 | 100 |
| 2 | 2 | 100 |
| 3 | 2 | 100 |
| Total | 6 | 100 |

chete-infected hosts within a day of attachment and become infectious to other hosts within another 3 to 5 days.

## DISCUSSION

The risk of acquiring infection by the agent of Lyme disease following a bite by an infected tick has been associated with the duration of attachment of the infecting tick (14, 16). All mice on which an infected nymph had fed for more than 48 h became infected. These observations are consistent with previous findings for laboratory-reared white-footed mice, hamsters, and rabbits (14, 16). Thus, we confirm that spirochetes are transmitted to mammalian hosts mainly after infected nymphal ticks have been attached to a host for 48 h or more.

The ability of partially fed nymphal *I. dammini* ticks to transmit Lyme disease infection remains unknown. A previous report stated that larval deer ticks that had fed partially on infected hosts failed to transmit spirochetal infection during refeeding (12). We found, however, that spirochetal infection is efficiently transmitted by partially fed nymphs. The duration of refeeding required for the partially fed nymphs to accomplish transmission is less than that required for infected flat nymphs. Indeed, spirochetes can be acquired by a feeding nymph as early as 8 h postattachment (21), and these partially fed nymphs effectively transmit spirochetal infection after refeeding. The effective duration of the interval between feedings exceeds 5 days. Thus, spirochetal infection transmitted by a partially fed nymphal tick occurs more rapidly than in the case of a nonfed tick.

The efficiency of transmission of spirochetes has been attributed to the magnitude of the spirochetal inoculum injected by a feeding tick (12, 17). Spirochete densities in partially fed larval deer ticks are said to be too sparse for efficient transmission (12). We found, however, that spirochetal infection is effectively transmitted by one partially fed nymph. In addition, spirochetes were transmitted by intradermal syringe injection of less than 10 cultured spirochetes (3). Thus, we suggest that the number of spirochetes injected by a partially fed nymph is sufficient to infect a mouse.

Transmission of spirochetal infection from partially fed nymphs to their hosts depends on the capacity of a tick to acquire spirochetes early in the feeding process as well as its ability to reattach to another host. We found that spirochetal infection is efficiently acquired within the first day of feeding and that ticks can reinfest new hosts. We found that nymphs do detach spontaneously from free-ranging mice in the laboratory, perhaps as frequently as 15% of the time. Indeed, about a tenth of questing nymphs in nature seem to be distended (24), and reattachment by partially fed subadult ticks commonly occurs (1, 9, 12).

Our observation of accelerated transmission by ticks that fed transiently on infected hosts suggests certain unexpected events in the life cycle of these spirochetes. We believe that this acceleration is not due to contaminative transmission because reattachment was delayed for several days. The previously observed phase of rapid multiplication (17) may be accompanied by spirochetal dissemination. Such disseminated infection normally is followed by a period of spirochete destruction that eliminates all but those spirochetes that survive in the lumen of the gut. Indeed, disseminated infections in nonfeeding ticks have occasionally been encountered during the course of fine-structural studies (6, 27). Such hemocelic spirochetes may become nonviable by the time that the tick has molted, and this persistence may reflect some failure of clearance by the inflammatory system of the tick. Lyme disease spirochetes, then, transiently disseminate soon after they are ingested by vector ticks.

Exposure to vector ticks infected by the agent of Lyme disease generally occurs during outdoor activities associated with recreation or employment (19, 22). In one study at a site at which Lyme disease was endemic, vector deer ticks were found on or near the well-maintained lawns of the home of a patient with Lyme disease (8). Spirochetal infection was detected in 33% of the nymphs and 55% of the adults. Indeed, vector ticks feeding on infected hosts may detach prematurely because of grooming or host-derived antitick immunity (4, 5, 25, 26). These partially fed ticks may already have acquired spirochetal infection and avidly seek other hosts. Pet ownership appears to be a risk factor for human Lyme disease (10, 23), and this may reflect contact with ticks that have detached from a cat or dog within the household. Although the natural frequency of such partially fed nymphs has not been determined, they may present a particularly great risk of transmission of spirochetes.

Taken together, these observations indicate that partially fed nymphs are able to reattach to another host and that Lyme disease spirochetes may be transmitted by partially fed nymphs more rapidly than by nymphs that have not already fed.

## ACKNOWLEDGMENTS

This work was supported in part by grant AI-29724 from the National Institutes of Health and by a scholarship from the National Defense Medical Center, Taipei, Taiwan, Republic of China.

## REFERENCES

1. **Balashov, Y. S.** 1972. Bloodsucking ticks (Ixodoida)—vectors of diseases of man and animals. Misc. Publ. Entomol. Soc. Am. **8**:301–305.
2. **Barbour, A. G., S. L. Tessier, and W. J. Todd.** 1983. Lyme disease spirochetes and ixodid tick spirochetes share a common surface antigenic determinant defined by a monoclonal anti-

body. Infect. Immun. **41**:795–804.
3. **Barthold, S. W.** 1991. Infectivity of *Borrelia burgdorferi* relative to route of inoculation and genotype in laboratory mice. J. Infect. Dis. **163**:419–420.
4. **Brossard, M., and V. Fivaz.** 1982. *Ixodes ricinus* L: mast cells, basophils and eosinophils in the sequence of cellular events in the skin of infested or re-infested rabbits. Parasitology **85**:583–592.
5. **Brown, S. J., and P. W. Askenase.** 1985. Rejection of ticks from guinea pigs by antihapten-antibody-mediated degranulation of basophils at cutaneous basophil hypersensitivity sites: role of mediators other than histamine. J. Immunol. **134**:1160–1165.
6. **Burgdorfer, W., S. F. Hayes, and D. Corwin.** 1989. Pathophysiology of the Lyme disease spirochete, *Borrelia burgdorferi*, in ixodid ticks. Rev. Infect. Dis. **11**(Suppl. 6):S1442–S1450.
7. **Donahue, J. G., J. Piesman, and A. Spielman.** 1987. Reservoir competence of white-footed mice for Lyme disease spirochetes. Am. J. Trop. Med. Hyg. **36**:92–96.
8. **Falco, R. C., and D. Fish.** 1988. Prevalence of *Ixodes dammini* near the homes of Lyme disease patients in Westchester County, New York. Am. J. Epidemiol. **127**:826–830.
9. **Gregson, J. D.** 1967. Observations on the movement of fluids in the vicinity of the mouthparts of naturally feeding *Dermacentor andersoni* Stiles. Parasitology **57**:1–8.
10. **Lane, R. S., and R. E. Lavoie.** 1988. Lyme borreliosis in California: acarological, clinical, and epidemiological studies. Ann. N.Y. Acad. Sci. **539**:192–203.
11. **Levine, J. F., M. L. Wilson, and A. Spielman.** 1985. Mice as reservoirs of the Lyme disease spirochete. Am. J. Trop. Med. Hyg. **34**:355–360.
12. **Piesman, J.** 1991. Experimental acquisition of the Lyme disease spirochete, *Borrelia burgdorferi*, by larval *Ixodes dammini* (Acari:Ixodidae) during partial bloodmeals. J. Med. Entomol. **28**:259–262.
13. **Piesman, J.** 1993. Standard system for infecting ticks (Acari: Ixodidae) with the Lyme disease spirochete, *Borrelia burgdorferi*. J. Med. Entomol. **30**:199–203.
14. **Piesman, J., T. N. Mather, R. J. Sinsky, and A. Spielman.** 1987. Duration of tick attachment and *Borrelia burgdorferi* transmission. J. Clin. Microbiol. **25**:557–558.
15. **Piesman, J., T. N. Mather, S. R. Telford III, and A. Spielman.** 1986. Concurrent *Borrelia burgdorferi* and *Babesia microti* infection in nymphal *Ixodes dammini*. J. Clin. Microbiol. **24**: 446–447.
16. **Piesman, J., G. O. Maupin, E. G. Campos, and C. M. Happ.** 1991. Duration of adult female *Ixodes dammini* attachment and transmission of *Borrelia burgdorferi*, with description of a needle aspiration isolation method. J. Infect. Dis. **163**:895–897.
17. **Piesman, J., J. R. Oliver, and R. J. Sinsky.** 1990. Growth kinetics of the Lyme disease spirochetes (*Borrelia burgdorferi*) in vector ticks (*Ixodes dammini*). Am. J. Trop. Med. Hyg. **42**:352–357.
18. **Schwan, T. G., W. J. Simpson, M. E. Schrumpf, and R. H. Karstens.** 1989. Identification of *Borrelia burgdorferi* and *B. hermsii* using DNA hybridization probes. J. Clin. Microbiol. **27**:1734–1738.
19. **Schwartz, B. S., and M. D. Goldstein.** 1990. Lyme disease in outdoor workers: risk factors, preventive measures, and tick removal methods. Am. J. Epidemiol. **131**:877–885.
20. **Shih, C. M., R. J. Pollack, S. R. Telford III, and A. Spielman.** 1992. Delayed dissemination of Lyme disease spirochetes from the site of deposition in the skin of mice. J. Infect. Dis. **166**:827–831.
21. **Shih, C. M., and A. Spielman.** Submitted for publication.
22. **Smith, P. F., J. L. Benach, D. J. White, D. F. Stroup, and D. L. Morse.** 1988. Occupational risk of Lyme disease in endemic areas of New York state. Ann. N.Y. Acad. Sci. **539**:289–300.
23. **Steere, A. G., T. F. Broderick, and S. E. Malawista.** 1978. Erythema chronicum migrans and Lyme arthritis: epidemiological evidence for a tick vector. Am. J. Epidemiol. **108**:312–321.
24. **Telford, S. R., III.** 1993. Personal communication.
25. **Trager, W.** 1939. Acquired immunity to ticks. J. Parasitol. **25**:57–81.
26. **Wikel, S. K., and J. R. Allen.** 1982. Immunological basis of host resistance to ticks, p. 169–196. *In* F. D. Obenchain and R. Galun (ed.), Physiology of ticks. Pergamon, Oxford.
27. **Zung, J. L., S. Lewengrub, M. Rudzinska, A. Spielman, S. R. Telford III, and J. Piesman.** 1989. Fine structural evidence for the penetration of the Lyme disease spirochete *Borrelia burgdorferi* through the gut and salivary tissues of *Ixodes dammini*. Can. J. Zool. **67**:1737–1748.