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Simultaneous Transmission of *Borrelia burgdorferi* and *Babesia microti* by Individual Nymphal *Ixodes dammini* Ticks

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Nymphal *Ixodes dammini* ticks, selected from a group of ticks in which 22 of 31 (71%) contained dual *Borrelia burgdorferi* and *Babesia microti* infections, simultaneously transmitted *B. burgdorferi* and *B. microti* to 4 of 7 (57%) hamsters exposed to individual ticks.

Simultaneous infection with the etiologic agents of Lyme disease (*Borrelia burgdorferi*) and human babesiosis (*Babesia microti*) has been documented in humans (2, 4, 9), rodent reservoirs (1), and nymphal *Ixodes dammini* ticks (7). Presumably, dual human infection with these two agents could result from the bite of a single tick simultaneously infected with both pathogens. However, we lack formal documentation of simultaneous transmission of *B. microti* and *B. burgdorferi* by individual ticks. Accordingly, we sought to experimentally demonstrate simultaneous *Babesia* and *Borrelia* transmission by individual nymphal *I. dammini* ticks.

All ticks used in this study were derived from adult *I. dammini* ticks collected from vegetation on Great Island, Yarmouth, Mass. The tick colony was maintained at 21°C and 97% relative humidity. Our *I. dammini* colony was previously shown to be free of transovarially acquired spirochetes (6). Golden Syrian female hamsters (50 to 150 g; Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were used as hosts in this study. The *B. burgdorferi* strain used in these experiments (JD1) originated from 15 to 20 nymphal *I. dammini* ticks collected on Crane's Beach, Ipswich, Mass. These field-collected nymphs fed on a laboratory-reared white-footed mouse which subsequently served as a host for xenodiagnostic larval *I. dammini* as previously described (3). These ticks molted and fed as nymphs on a hamster. This hamster served as a host for the ticks used in this study. The JD1 strain was identified as *B. burgdorferi* based on its ability to bind monoclonal antibody H5332 (6). The *B. microti* strain used (GI) was isolated and maintained as previously described (5).

A xenodiagnostic procedure was used to detect *Borrelia* and *Babesia* infection in hamsters (3, 6). In this procedure, colony-derived larval *I. dammini* ticks were allowed to feed to repletion on test hamsters. The larvae molted to become nymphs. The resultant xenodiagnostic nymphs were then allowed to attach to noninfected hamsters for 54 h, after which they were removed from the hosts. The salivary glands of these ticks were then dissected, treated with Feulgen reagent, and examined for salivarian *B. microti* as previously described (5). The midgut and other tissues from these same ticks were examined by a direct fluorescent-antibody test for *B. burgdorferi* as previously described (7, 8).

In a preliminary experiment, a hamster was exposed simultaneously to infestation with *B. microti*- and *B. burgdorferi*-infected nymphs. A total of eight nymphs from a group of *B. microti*-infected nymphs (50% infected) and five nymphs from a group of *B. burgdorferi*-infected nymphs (90% infected) were allowed to infest this hamster. Six replete nymphs were recovered. At 28 days post-infectious exposure, larval *I. dammini* ticks were fed on this hamster. About 50 of these larvae molted to become nymphs, and a sample was examined for *Babesia* and *Borrelia* infection. Of 31 nymphs examined, 8 were infected with *B. microti* alone, 1 was infected with *B. burgdorferi* alone, and 22 (71%) were infected with both organisms.

We used the remaining not previously examined nymphal *I. dammini* ticks from this group of dually infected ticks to determine whether individual nymphs could transmit *B. burgdorferi* and *B. microti* simultaneously. At 3 to 4 months postmolting, individual nymphs were allowed to feed on hamsters (one nymph per hamster). Hamsters from which replete nymphs were not recovered were discarded from the experimental analysis. A total of seven hamsters were successfully fed upon by individual nymphs. These seven hamsters were exposed to xenodiagnostic larvae at 3 to 4 weeks post-nymphal exposure. Xenodiagnostic ticks were subsequently examined for *Borrelia* and *Babesia* infection. Of the seven hamsters tested, four (57%) proved to be dually infected (Table 1); two hamsters were infected with *B. burgdorferi* alone, and one hamster was infected with *B. microti* alone. Thus, most of the nymphal *I. dammini* ticks simultaneously transmitted *B. burgdorferi* and *B. microti*.

The observations that human residents of endemic regions acquired concurrent infection with *B. burgdorferi* and *B. microti* (2, 4, 9) suggested that nymphal *I. dammini* ticks could transmit both organisms simultaneously. Moreover,

TABLE 1. Simultaneous transmission of *B. burgdorferi* and *B. microti* by individual nymphal *I. dammini* ticks

Host[a]	Proportion (no. infected/no. examined) of xenodiagnostic ticks infected by:		Dual transmission
	B. burgdorferi	*B. microti*	
1	14/15	14/15	Positive
2	13/15	8/15	Positive
3	11/12	0/12	Negative
4	2/2	1/2	Positive
5	8/15	0/15	Negative
6	0/11	10/11	Negative
7	15/18	16/18	Positive

[a] Each hamster was fed upon by one nymph.

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field-collected nymphal *I. dammini* ticks were frequently infected with both agents in two endemic locations in Massachusetts (7). The present study provides definitive evidence that infection in ticks with one of these agents does not preclude transmission of the other agent. This evidence adds emphasis to the recommendation that clinicians treating patients who have either Lyme disease or babesiosis should consider the possibility of dual human infection in regions where both *B. burgdorferi* and *B. microti* are endemic.

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LITERATURE CITED

1. **Anderson, J. F., R. C. Johnson, L. A. Magnarelli, F. W. Hyde, and J. E. Myers.** 1986. *Peromyscus leucopus* and *Microtus pennsylvanicus* simultaneously infected with *Borrelia burgdorferi* and *Babesia microti*. J. Clin. Microbiol. **23**:135–137.
2. **Benach, J. L., J. L. Coleman, G. S. Habicht, A. MacDonald, E. Grunwaldt, and J. A. Giron.** 1985. Serological evidence for simultaneous occurrences of Lyme disease and babesiosis. J. Infect. Dis. **152**:473–477.
3. **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.
4. **Marcus, L. C., A. C. Steere, P. H. Duray, A. E. Anderson, and E. B. Mahoney.** 1985. Fatal pancarditis in a patient with coexistent Lyme disease and babesiosis. Ann. Intern. Med. **103**:374–376.
5. **Piesman, J., S. J. Karakashian, S. Lewengrub, M. A. Rudzinska, and A. Spielman.** 1986. Development of *Babesia microti* sporozoites in adult *Ixodes dammini*. Int. J. Parasitol. **16**:381–385.
6. **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.
7. **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.
8. **Steere, A. C., R. L. Grodzicki, A. N. Kornblatt, J. E. Craft, A. G. Barbour, W. Burgdorfer, G. P. Schmid, E. Johnson, and S. E. Malawista.** 1983. The spirochetal etiology of Lyme disease. N. Engl. J. Med. **308**:733–742.
9. **Steketee, R. W., M. R. Eckman, E. C. Burgess, J. N. Kuritsky, J. Dickerson, W. L. Schell, M. S. Godsey, and J. P. Davis.** 1985. Babesiosis in Wisconsin. A new focus of disease transmission. J. Am. Med. Assoc. **253**:2675–2678.