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NOTES

Concurrent *Borrelia burgdorferi* and *Babesia microti* Infection in Nymphal *Ixodes dammini*†

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***Ixodes dammini* nymphs were examined for evidence of concurrent infection with *Borrelia burgdorferi* and *Babesia microti*. A total of 19 nymphs (18.6%) from Nantucket Island were simultaneously infected, as were 24 nymphs (8.2%) from Naushon Island. These observations are consistent with a common reservoir host for both *I. dammini*-transmitted pathogens.**

Concurrent human infection by two *Ixodes dammini*-borne pathogens, *Borrelia burgdorferi* and *Babesia microti*, has recently been described (5, 8, 12). Such dual infection could derive from the bites of two different ticks, each infected with one etiologic agent, or from the bite of a single tick simultaneously infected with both pathogens. Simultaneous *Babesia* and spirochetal infections occur in rodent reservoir hosts (2), thereby providing the opportunity for larval *I. dammini* to acquire both agents simultaneously and pass both pathogens transstadially to the vector nymphal stage. Accordingly, we collected questing nymphal *I. dammini* in sites endemic for human babesiosis and Lyme disease and determined whether these ticks were infected by *B. microti* and *B. burgdorferi*.

Nymphal *I. dammini* ticks were collected from May through September 1985. Questing ticks were collected by dragging a corduroy or flannel flag through vegetation. Ticks were collected on the University of Massachusetts Field Station-Nantucket Island, as well as on Naushon Island, Mass. These study sites have been previously described (9). Nymphs collected from these study sites have been found to harbor *B. microti*, as well as *B. burgdorferi* (Piesman et al., Acta Trop., in press).

All field-collected *I. dammini* were taken to the laboratory and held at 21°C and 95% relative humidity for <1 week. Nymphs were then allowed to attach to noninfected, female, golden Syrian hamsters weighing 50 to 150 g. All hamsters were purchased from Charles River Breeding Laboratories, Wilmington, Mass. Nymphs were allowed to feed on hamsters for 54 h, at which time all ticks were removed. Individual nymphs were placed on glass microscope slides in 1 drop of phosphate-buffered saline. The salivary glands from each tick were dissected and transferred to 1 drop of phosphate-buffered saline on a gelatin-coated slide. Salivary gland preparations were air dried and fixed in methanol. These salivary gland preparations were then subjected to the Feulgen reaction to detect *B. microti* parasites. The Feulgen reaction, modified for the detection of *B. microti*, has been described before (Piesman et al., in press).

The remaining internal organs of each nymph (midgut, central ganglion, malpighian tubules, etc.) were dissected away from the cuticle in the original drop of saline. These tissues were mounted and squashed with a cover slip. The cover slip was then removed, and the slides were air dried, fixed in acetone, and stored at −20°C. Subsequently, these slides were treated with a 1:100 dilution of fluorescein-isothiocyanate-conjugated antibodies. The polyclonal antibody was produced in rabbits immunized with the Guilford strain of *B. burgdorferi*, kindly supplied by Alan Steere. Antibody-treated slides were processed and examined for spirochetes as previously described (11).

We examined the association between babesial and spirochetal infections in nymphal *I. dammini* collected on Nantucket and Naushon Islands. Of the 102 nymphs collected on Nantucket Island, 19 (18.6%) were simultaneously infected with *B. microti* and *B. burgdorferi* (Table 1). Similarly, a total of 24 out of 293 nymphs (8.2%) from Naushon Island were dually infected. There was a significant association between babesial and spirochetal infections in nymphal *I. dammini* on Nantucket Island (chi-square = 5.65, $P < 0.02$) and Naushon Island (chi-square = 17.14, $P < 0.001$). The number of observed ticks with dual infection was greater than the chi-square expected value on both islands (dually infected ticks: Nantucket = 19 observed, chi-square expected = 13.5; Naushon = 24 observed, chi-square expected = 12.5).

The positive association between babesial and spirochetal infections in nymphal *I. dammini* is consistent with a common reservoir host for both agents. Simultaneous infection with *B. microti* and *B. burgdorferi* has been described for *Peromyscus leucopus* and *Microtus pennsylvanicus* (2). Moreover, *P. leucopus* is thought to be the primary reservoir host for *B. microti* (10) and *B. burgdorferi* (1, 7). The dually infected nymphal *I. dammini* observed in our study probably acquired their infections by feeding as larvae on *P. leucopus*, the principal host for immature *I. dammini* (9).

The spirochetes we observed in *I. dammini* ticks are presumably *B. burgdorferi* since this species is the only spirochete known to naturally infect *I. dammini*. However, the recently available genetic (3, 6) and monoclonal antibody probes (4), which are diagnostic for *B. burgdorferi*, should

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TABLE 1. Prevalence of *B. burgdorferi*, *B. microti*, and concurrent infection of both pathogens in questing nymphal *I. dammini* collected from Nantucket and Naushon Islands, May to September 1985

Location	No. of nymphs examined	No. of nymphs infected by:		
		B. burgdorferi alone	*B. microti* alone	Both pathogens
Nantucket	102	13	24	19
Naushon	293	52	24	24

be applied to spirochetes derived from natural populations of *I. dammini* to confirm that *B. burgdorferi* is indeed the sole spirochete infecting these tick populations. Such studies were considered beyond the scope of the present investigation.

Our observations have established that concurrent infection with *B. microti* and *B. burgdorferi* commonly occurs in field-collected nymphal *I. dammini*. Demonstration of simultaneous transmission of both agents by individual ticks, however, is still lacking. Laboratory experiments with dually infected ticks should be performed to determine whether the presence of one *I. dammini*-borne pathogen interferes with the transmission of the other pathogen. Our results confirm the recommendation (2, 5, 8) that patients presenting with symptoms of either babesiosis or Lyme disease should be examined for evidence of infection by both *B. microti* and *B. burgdorferi*.

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