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Comparative prevalence of *Babesia microti* and *Borrelia burgdorferi* in four populations of *Ixodes dammini* in eastern Massachusetts

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Summary

We determined the prevalence of *Babesia microti* and *Borrelia burgdorferi* in four populations of *Ixodes dammini* in eastern Massachusetts. The Feulgen's reaction was more sensitive than the Giemsa method for detecting salivarian *Babesia*. A combination of darkfield and direct-fluorescent-antibody examination proved more sensitive than either method alone for detecting spirochetal infection. The prevalence of spirochetes was greater than the prevalence of *Babesia* in each of the tick populations studied. Overall, 24% of nymphs and 47% of adults examined were infected with spirochetes; in contrast, 11% of nymphs and 14% of adults were infected with *Babesia*. The difference between the spirochetal and babesial prevalence was greatest in the most recently infested site. The rising incidence of Lyme disease, as compared to the stable incidence of human babesiosis, may result from the relatively greater abundance of *Bo. burgdorferi* infected *I. dammini* ticks in newly infested locations.

Key words: *Babesia microti; Borrelia burgdorferi;* ticks; *Ixodes dammini;* salivary glands.

Introduction

Although the etiologic agents of Lyme disease *(Borrelia burgdorferi)* and human babesiosis *(Babesia microti)* are transmitted by the same tick species *(Ixodes dammini)* in the northeastern United States, the epidemiologic patterns

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of these two diseases differ markedly. Human babesiosis has been limited to sharply defined foci in coastal Massachusetts and New York; about 10 clinical cases per year have been recognized on a regular basis since 1970 (Dammin et al., 1981). In contrast, Lyme disease seems to affect ever increasing numbers of people over an expanding geographic range (Anon., 1984, 1985).

The discrepant epidemiology of human babesiosis and Lyme disease may result from differences in prevalence of the two etiologic agents in tick populations. Greater prevalence of one agent could lead to increased intensity of transmission. Accordingly, we compared the prevalence of *Bo. burgdorferi* and *Ba. microti* in four local populations of *I. dammini* in eastern Massachusetts.

Materials and Methods

Babesia detection

Salivary glands were dissected into a drop of PBS and placed onto gelatin coated slides. All slides were air-dried and fixed in methanol for 10 min. Subsequently, glands were stained with Giemsa's or Feulgen's stain and examined for *Ba. microti* parasites. Giemsa's stained glands were prepared as previously described (Piesman and Spielman, 1980, 1982). Feulgen's stained glands were treated as described by Blewett and Branagan (1973) with slight modifications. Briefly, glands were placed into 1N HCl at room temperature for 5 min, 1N HCl at 60°C for 12 min, washed in distilled water and stained in Schiff's reagent for 90 min. After washing in tap water, as well as 70% and 95% ethanol, glands were counterstained in 0.2% Fast Green in 95% alcohol for 1–2 min. Slides were then rinsed in 95% alcohol, transferred to absolute ethanol, then xylene and finally mounted in permount.

For experiments requiring *Ba. microti* infected ticks, larval *I. dammini* were fed on hamsters infected with the "GI" strain isolated from a patient who acquired babesiosis on Nantucket Island during 1982. Infected ticks were held at 21°C. Laboratory reared *I. dammini* ticks were originally collected on Great Island, Massachusetts and maintained as previously described (Spielman et al., 1979).

Spirochete detection

In order to detect *Bo. burgdorferi* spirochetes in *I. dammini*, the intestine and other tissues of ticks were dissected into a drop of PBS on a microscope slide, covered with an 18 mm circular coverslip, squashed, and examined for spirochetes under darkfield illumination at 640 times magnification. After darkfield examination, coverslips were removed, slides air-dried, acetone fixed, and stored at −24°C. In an additional attempt to visualize spirochetes, slides were flooded with fluorescein isothiocyanate conjugated antibodies produced in rabbits inoculated with *Bo. burgdorferi*, and examined as previously described (Burgdorfer et al., 1982; Steere et al., 1983).

Field survey

Ticks were collected by dragging flannel or corduroy "flags" over vegetation in 4 previously described locations in eastern Massachusetts: Nantucket Is., Great Is., Naushon Is., and Crane's Beach (Piesman and Spielman, 1979: Wilson et al., 1984, 1985). Nymphal *I. dammini* were collected from June–September, 1984 and adult *I. dammini* were collected from October–December, 1984.

Results

In a preliminary experiment, we compared Giemsa's stain and Feulgen's reaction as methods for detecting salivarian *Ba. microti*. Batches of nymphal *I. dammini*, previously fed as larvae on a *Ba. microti* infected hamster, were allowed to feed on a noninfected hamster for 54 h before dissection. In one batch

Table 1. Comparison of Giemsa and Feulgen's methods for detection of salivarian *Babesia microti*

Staining method	Heavily infected ticks*	Lightly infected ticks**
	No. examined % (+)	No. examined % (+)
Feulgen's	18 (100.0)	21 (76.2)
Giemsa	17 (88.2)	21 (23.8)

* Mean = 19.9 infected acini
** Mean = 3.2 infected acini

Table 2. Demonstration of salivarian *Babesia microti* in Feulgen's preparations at various times post-tick attachment

Hours post-attachment	No. nymphs examined	No. positive (%)	Mean no. acini infected per infected tick
0	15	9 (60)	4.8
24	16	10 (63)	6.9
36	17	13 (77)	7.3
48	14	11 (79)	8.5
54	15	15 (100)	19.9
60	13	9 (69)	12.2
72	20	15 (75)	7.9
96*	18	7 (39)	2.4

* replete at 96 h

of nymphs developing intense salivarian infection, 100% of Feulgen's treated glands and about 90% of Giemsa's stained preparations were judged to be infected (Table 1). In a group of lightly infected nymphs, however, significantly more Feulgen's prepared glands were diagnosed as infected, as compared to Giemsa's stained glands (Chi-square = 11.52; $P<0.005$). Consequently, we selected the Feulgen's reaction as our method of choice for diagnosing salivarian *Ba. microti.*

We then determined when following nymphal attachment, salivarian *Babesia* can be detected. A group of heavily infected nymphs were examined at about 12-h intervals post-attachment to noninfected hamsters. The proportion of nymphs with demonstrable infection, as well as the number of infected acini, were significantly greater at 54 h post-attachment than all other time intervals (proportion infected, Chi-square = 7.45, $P<0.01$; number acini infected, $F = 11.17$, $P<0.005$) (Table 2). Interestingly, replete ticks had the lowest intensity of infection, even less than nonfed ticks. We adopted 54 h post-attachment as the optimal time for detecting *Babesia* in nymphal salivary glands. In addition, a group of field collected female *I. dammini* were placed on a rabbit's ear; 12 females were removed daily, their salivary glands dissected and examined using

Table 3. Comparison of the darkfield (DF) and direct-fluorescent antibody (DFA) tests for detecting *Borrelia burgdorferi* in partially fed and nonfed (flat) *Ixodes dammini*

Stage	Feeding	No. examined	% infected		
			DF	DFA	Combined**
Nymph	Fed*	46	11	15	15
	Flat	182	21	20	22
Female	Fed*	23	0	35	35
	Flat	120	35	49	49
Male	Flat	120	38	45	46

* Nymphs fed on hamsters for 54 h; females fed on rabbits for 4 days.
** Combined = Positive on at least one of the two tests performed (DF and DFA).

the Feulgen's reaction to detect salivarian *Babesia*. The greatest proportion of infected ticks was observed at four days post-attachment (42%), as opposed to two days (25%), three days (17%), or five days (8%). Consequently, adult salivary glands were examined for *Babesia* at four days post-attachment to laboratory rabbits.

Two methods, darkfield (DF) and direct-fluorescent-antibody (DFA) examination, were compared for sensitivity in detecting Lyme disease spirochetes in field collected *I. dammini*. Individual nymphs and adults were examined in a nonfed (flat) or partially fed state; the DFA method was significantly more sensitive than the DF method in fed ticks (Chi-square = 5.85; $P < 0.05$). There was no significant difference between the two methods in flat ticks (Chi-square = 2.85, $P > 0.05$) (Table 3). Of the 491 ticks examined, three were positive on DF and negative on DFA, but 22 ticks were negative on DF and positive on DFA. In our field survey, we examined flat ticks by both the DF and DFA methods for spirochetal infection.

Babesia and spirochetes were detected in all four local populations of *I. dammini* studied (Tables 4 and 5). Spirochetal prevalence was greater than that of *Babesia*, both in nymphs and adults. Overall, 24% of nymphs and 47% of adults contained spirochetes; in contrast, a significantly lesser proportion of nymphs (11%) (Chi-square = 20.11, $P < 0.001$) and adults (14%) (Chi-square = 79.07, $P < 0.001$) contained salivarian *Babesia*. Great Island ticks were most frequently infected with *Babesia* and spirochetes; the prevalence of both agents in Naushon Island ticks was relatively depressed. The difference between spirochetal and babesial prevalence was most significant in the Crane's Beach population (Chi-square = 26.76, $P < 0.001$), where infection by *Ba. microti* was rare. Spirochetal infection rates were significantly higher in adults than in nymphs in all locations (Chi-square = 21.25, $P < 0.001$); babesial infection was more prevalent in adults than in nymphs, but this difference in infection rates was not significant (Chi-square = 2.36, $P > 0.05$).

Table 4. Comparative prevalence of *Babesia microti* and *Borrelia burgdorferi* in nymphal *Ixodes dammini* collected from 4 locations in eastern Massachusetts

Location	*Babesia**		Spirochete**	
	No. examined	% infected	No. examined	% infected
Great Is.	152	16	38	29
Nantucket Is.	122	16	48	25
Naushon Is.	195	8	48	15
Crane's Beach	118	2	48	27

* Nymphs examined for *Babesia* after feeding on hamsters for 54 h.
** Nymphs examined in nonfed (flat) state for spirochetes.

Table 5. Comparative prevalence of *Babesia microti* and *Borrelia burgdorferi* in adult *Ixodes dammini* collected from 4 locations in eastern Massachusetts

Location	Sex	*Babesia**		Spirochete**	
		No. examined	% infected	No. examined	% infected
Great Is.	Female	65	37	30	63
	Male	126	10	30	67
Nantucket Is.	Female	62	11	30	67
	Male	17	6	30	50
Naushon Is.	Female	30	17	30	27
	Male	30	13	30	27
Crane's Beach	Female	57	2	30	40
	Male	12	0	30	37

* Adults examined for *Babesia* after 4 days feeding on rabbits.
** Adults examined for spirochetes in nonfed (flat) state.

Discussion

Although *Bo. burgdorferi* was abundantly prevalent in all 4 tick populations studied, *Ba. microti* rarely infected ticks collected from the Crane's Beach location. Crane's Beach is unique among the four locations in two important respects: 1. It is a newly infested location (Spielman et al., 1985). 2. It is a mainland location. All other locations studied were islands. Both these factors may have contributed to the infrequent prevalence of *Babesia* in Crane's Beach-ticks. Birds may be the most likely candidates for introducing *I. dammini* into new locations, since these hosts are apparently capable of sustaining spirochetal infection (Anderson and Magnarelli, 1984). Thus, larval *I. dammini* may acquire *Bo. burgdorferi* infection from the phoretic avian hosts which introduce them into new locations. On the other hand, *Ba. microti* probably does not infect

birds; therefore introduction of this protozoan pathogen into new locations may be impeded as compared to spirochetal introduction. The fact that Crane's Beach is a mainland location may also serve to reduce babesial prevalence. In fact, a preliminary survey of neighboring Hog Is., presumably infested with *I. dammini* simultaneous with infestation of Crane's Beach, revealed more *Ba. microti* infection in nymphs (3/23 = 13% infected) than the 2% level observed in Crane's Beach. Islands, because of their paucity of mammalian fauna (Healy et al., 1976; Piesman and Spielman, 1979), may be predisposed to intense transmission of *Ba. microti* since island ticks feed primarily on *Peromyscus leucopus,* the principal reservoir hosts of *Ba. microti* (Spielman et al., 1981).

Lyme disease spirochetes generally were more prevalent than were *Babesia* in the *I. dammini* populations studied. Some aspect of the vector-pathogen-reservoir host interactions must be more efficient in *Bo. burgdorferi* development and transmission than in *Ba. microti* transmission. Both agents are efficiently acquired and transmitted by *I. dammini* in our laboratory. However, the variety of reservoir hosts capable of sustaining spirochetes seems greater than in the case of *Ba. microti* infection. Moreover, duration of intense infection in hosts may span a longer period in reservoirs carrying spirochetes than in those carrying *Babesia.* These factors may partially explain the difference in spirochetal and babesial infection rates in vector tick populations.

The morphology of Feulgen's stained salivary glands infected with *Ba. microti* closely resembles descriptions of salivary glands infected with *Theileria* (Blewett and Branagan, 1973; Walker et al., 1981). In the laboratory, the Feulgen's reaction may be used as a routine method for selecting *Babesia* infected ticks for ultrastructural studies. Our attempts to demonstrate *Ba. microti* sporozoites in Feulgen's nonreactive salivary glands were invariably unsuccessful; in contrast, *Ba. microti* sporozoites matching those described by Karakashian et al. (1983) were readily observed in Feulgen's reactive glands. The sporozoites of the "Nuttallia-like" parasite *Ba. microti* resemble *Theileria* (Fawcett et al., 1982) more closely than *Babesia* (Moltmann et al., 1982). Moreover, sporozoites derived from Feulgen's reactive nymphal and adult *I. dammini* salivary glands proved infectious when inoculated into laboratory rodents (unpublished observations). The Feulgen's reaction is a more sensitive technique than Giemsa's stain, previously used to demonstrate *Ba. microti* in *I. dammini* salivary glands (Piesman and Spielman, 1980).

Both the darkfield method (DF) and direct-fluorescent-antibody method (DFA) for detecting *Bo. burgdorferi* spirochetes are useful in nonfed (flat) ticks (Burgdorfer et al., 1982; Steere et al., 1983). However, DFA is more sensitive than DF in engorged ticks. The decreased sensitivity of DF in fed ticks may result from the obscuring effect of bloodmeal contents.

Emphasis has been placed on the role of nymphal *I. dammini* as vectors of human babesiosis and Lyme disease, since most patients experience onset of illness during the months when nymphs generally feed (Ruebush et al., 1981;

Steere et al., 1981). Adult ticks, moreover, can more readily be detected and promptly removed than can the smaller nymphs. Adult *I. dammini* do attach to people, however, and may occasionally transmit *Ba. microti* or *Bo. burdgorferi* (Schulze et al., 1985). In our study, spirochetal prevalence was twice as high in adults as compared to nymphs. Unlike spirochetes, *Babesia* was not uniformly more prevalent in adults than in nymphs. Since adult *I. dammini* feed principally on white-tailed deer, a host which is apparently resistant to *Ba. microti* infection (Piesman et al., 1979), the presence of *Babesia* in adult salivary glands may not be important in the enzootic cycle of *Ba. microti.* In contrast, white-tailed deer may become infected with *Bo. burgdorferi* (Bosler et al., 1983). Thus, infection of adult *I. dammini* with spirochetes could be important in the maintenance of enzootic *Bo. burgdorferi.*

Discovery of the spirochetal etiology of Lyme disease (Burgdorfer et al., 1982) led to an increased effort to document cases of human infection with *Bo. burgdorferi.* Subsequently, the incidence of Lyme disease has increased rapidly throughout the United States (Anon., 1984, 1985), while the incidence of human babesiosis has been relatively constant (Dammin et al., 1981). The prevalence of spirochetes in ticks was greater than that of *Babesia* in all 4 locations studied, especially the newly infested site Crane's Beach. Residents of newly infested locations, e.g. Westchester Co., New York, may be exposed to substantial risk of Lyme disease transmission, while risk of infection with *Babesia,* for the present, remains negligible.

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