Geographical and Temporal Distribution of Babesial Infection in Connecticut

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Human babesiosis was first recognized in Connecticut in 1989, nearly 15 years after Lyme disease, a similarly transmitted spirochetosis, was detected in the state. To determine the seroprevalence for the babesial pathogen and whether it was recently introduced, we used an indirect immunofluorescence assay to test for *Babesia microti* antibody in 1,285 Connecticut residents. Four groups were studied: I, people seropositive for Lyme disease, tested from 1986 to 1989; II, randomly selected outpatients tested in 1989; III, college students residing in Connecticut, tested from 1959 to 1989; and IV, healthy people without tick exposure or Lyme disease, tested in 1989. *Babesia* seropositivity was significantly higher in group I (9.5%; $n = 735$) than in groups II (2.6%; $n = 304$, $P < 0.0001$) and III (1.0%; $n = 206$, $P < 0.0001$) but not group IV (2.5%, $n = 40$). *Babesia* seropositivity for group I ranged from 9.2 to 10.2% between 1986 and 1989, and *Babesia* seropositivity for group III ranged from 0% between 1959 and 1985 to 2.9% between 1986 and 1989. There is a considerable risk of babesial infection among residents of the Connecticut mainland who are seropositive for Lyme disease, a risk that appears to have remained constant over the past 5 years.

Babesiosis is a malarialike infection recognized since biblical times as murrain in cattle but only recently as a zoonotic pathogen of humans (6, 7, 13, 15). Although human infections have been attributed to diverse species of *Babesia*, a parasite of rodents, *Babesia microti*, appears to be the common agent of human disease. Clinical manifestations include fever, anorexia, malaise, headache, fatigue, chills, and anemia (6, 7, 13, 15). Infection is generally asymptomatic or self-limited, but it can be severe or fatal in elderly, asplenic, or immunocompromised hosts (8, 12, 24, 25, 27). *B. microti* is transmitted by the same group of *Ixodes* ticks that transmit *Borrelia burgdorferi*, the causative agent of Lyme disease (29). In the northeastern United States, *B. microti* and *B. burgdorferi* are transmitted by the tick *Ixodes dammini* (29). Coinfection in the vector as well as in the rodent reservoir host (*Peromyscus leucopus*) is common (2, 21).

Human infection by *B. microti* has been reported most frequently from the chain of islands along the southern coast of New England including Nantucket, Martha's Vineyard, Block Island, and Long Island (6, 7, 13, 15). Few cases were reported from the mainland until 1989, when clusters of cases were recognized in coastal southeastern Connecticut, nearly 15 years after epidemic Lyme disease in this area was first described (11).

The apparent novelty of this mainland focus of babesiosis suggests that the infection became established in Connecticut more recently than did Lyme disease. To examine this hypothesis, we analyzed the prevalence of babesial antibody in diverse groups of Connecticut residents to determine whether (i) residents exposed to the agent of Lyme disease

are more frequently exposed to babesial infection than are other individuals, (ii) babesial antibody prevalence is highest in residents of southeastern Connecticut, and (iii) babesial seropositivity has increased in recent years.

MATERIALS AND METHODS

Study population. This study was reviewed and approved by the Institutional Review Board of the University of Connecticut School of Medicine. Four study groups of Connecticut residents were tested for antibody against *B. microti*. Group I consisted of 735 people tested for Lyme disease between 1986 and 1989 at The University of Connecticut Health Center and found to be seropositive. The Health Center microbiology laboratory serves as a Lyme disease reference testing center for the entire state of Connecticut; since 1986, aliquots of all sera positive for Lyme disease antibody have been stored at -70°C in the laboratory. Group II included 304 patients seeking medical care for reasons other than Lyme disease during the winter of 1989 at a family practice office in Lyme, Connecticut (group IIa) or at the Health Center (group IIb). Group III consisted of 206 healthy Yale University undergraduates who resided in Connecticut prior to entering college between 1959 and 1989. Group IV consisted of 40 healthy adults residing in the greater Hartford, Conn., area who had no history of tick exposure or clinical Lyme disease. Sera were tested for babesial antibody by using an indirect immunofluorescence antibody test (IFA) and for Lyme disease antibody by using an enzyme-linked immunosorbent assay (ELISA) following filtration of the serum samples through a 0.45- μm -pore-size micropore filter.

IFA for antibody to *B. microti*. A standard IFA was used to

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detect antibody to *B. microti* (5). Antigen for the IFA was derived from the GI strain of *B. microti* that was kept in continuous passage in female golden hamsters by monthly intraperitoneal inoculation combined with a vector passage every 4 months (22). When at least 40% of erythrocytes were parasitized, 1 ml of blood was collected in a heparinized syringe by cardiac puncture following halothane anesthesia. The blood was centrifuged at $500 \times g$ for 5 min, the plasma was removed, and the packed cells were diluted in 10 ml of phosphate-buffered saline (PBS) (pH 7.6). The cells were washed five times in PBS and resuspended in PBS so that thick smears contained 100 to 500 piroplasms per field ($\times 400$). A 2.5- μ l drop of cell suspension was added to each well of a 12-well glass IFA slide, which then was permitted to dry at 23°C, dated, and stored in a desiccator at 4°C until use. Such slides were used within 1 month of preparation.

For IFA testing, slides were permitted to come to room temperature. Test sera were diluted 1:32 in PBS, and 20 μ l of the dilution was added to a slide well. Each slide was incubated for 30 min at 35°C, washed three times with agitation in PBS, and allowed to air dry. A 20- μ l drop of goat anti-human immunoglobulin labeled with fluorescein isothiocyanate (Kirkegaard and Perry, Gaithersburg, Md.) diluted in PBS and Evans blue (final concentration, 0.0005%) was added to each well and permitted to incubate for 30 min at 35°C. The slide was again washed three times with agitation in PBS, air dried, mounted with buffered glycerin, and covered with a coverslip. A Leitz fluorescence microscope with a 63 $\times$ oil immersion objective and 10 $\times$ oculars, for a total magnification of 630 $\times$, was used to examine the test slides. Controls used with each run included serum from a patient with babesiosis confirmed with a blood smear (positive control), serum from a healthy patient (negative control), and PBS. Reactions were graded on a scale of 0 to 4+. All specimens with a 1+ to 4+ reaction were retested at dilutions of 1:64. A positive serum sample was defined as one reacting at 1:64.

ELISA for *B. burgdorferi*. Seroreactivity to the agent of Lyme disease was determined by using a previously reported ELISA (16). Fifty microliters of a standardized spirochete suspension (strain 2591; Connecticut Agricultural Experiment Station, New Haven, Conn.) was added to alternate wells of flat-bottom microdilution plates (NUNC Immunoplate; Marsh, Rochester, N.Y.). In the other wells, 50 μ l of PBS was added as a control for nonspecific binding. Plates were dried overnight in a 37°C incubator and then stored in a plastic bag at -20°C until they were ready for use.

At the time of testing, 200 μ l of PBS containing 0.05% horse serum and 0.01% dextran sulfate was added to each well and incubated at 37°C for 1 h. The plates were washed six times with PBS-Tween 20, and 60 μ l of patient serum diluted 1:160 and 1:320 and the same amount of positive and negative control sera were added to different wells. The plates were incubated for 1 h at 37°C in a humid chamber and washed six times with PBS-Tween 20, and 60 μ l of peroxidase-labeled anti-human immunoglobulin G (1:5,000) or anti-human immunoglobulin M (1:5,000) was then added to each. Following an incubation for 1 h in a 37°C humid chamber, the plates were again washed six times with PBS-Tween 20. Sixty microliters of the substrate chromagen [equal volumes of 2,2'-azino-di-(3-ethylbenzthiazoline-sulfonate) and hydrogen peroxide; Kirkegaard and Perry] was added to each well.

Plates were read on a spectrophotometer at 414 nm when the optical density reading of the 1:160 dilution of the positive control minus the optical density reading of the nonspecific-binding well equaled 1.0 for immunoglobulin G

TABLE 1. Comparison of reactivities against *B. microti* antigen of sera that did and did not react against *B. burgdorferi* antigen

Type or source of serum (group ^a)	Yr of collection	Subjects	
		No. tested	% <i>Babesia</i> antibody positive ^b
<i>Borrelia</i> reactive (I)	1986-1989	735	9.5
Random (II)			
Family practice (IIa)	1989	100	4.0
University of Connecticut Health Center (IIb)	1989	204	2.0
College students (III)	1959-1989	206	1.0
<i>Borrelia</i> nonreactive (IV)	1989	40	2.5

^a Group I was reactive against *B. burgdorferi* antigen; groups II through IV were not.

^b Differences in *Babesia* reactivity were significant between group I and groups IIa, IIb, and III ($P < 0.0001$, $P = 0.042$, and $P < 0.0001$, respectively) but not between groups I and IV ($P = 0.10$).

or 0.5 for immunoglobulin M. A serum sample was considered positive when the net absorbance (the value for the antigen well minus the value for nonspecific-binding well) was 3 standard deviations or more above the mean absorbance of the negative control wells.

RESULTS

The sera of *B. burgdorferi* antibody-positive subjects (group I), of non-Lyme disease patients (groups II and III), and of those who had not been exposed to ticks or to Lyme disease (group IV) were compared for prevalence of *Babesia* antibody. Approximately 10% of group I sera, 3% of group II sera, 1% of group III sera, and 2% of group IV sera were reactive (Table 1). The prevalence of antibody against *B. microti* was significantly greater in group I than in group II (two-tailed Fisher exact test, $P < 0.0001$) and in group III (two-tailed Fisher exact test, $P < 0.0001$) but not in group IV (two-tailed Fisher exact test, $P = 0.10$). We concluded that people who have been infected with *B. burgdorferi* are at increased risk of infection with *B. microti* and that approximately 1 in 10 of these *B. burgdorferi*-infected Connecticut residents have been exposed to this piroplasm.

To determine whether the risk of acquiring babesial infection for Connecticut residents has increased during the past 30 years, we compared *Babesia* seroprevalence according to date of collection. The percentage of reactive sera from randomly chosen college students (group III) increased from 0% between 1959 and 1963 to nearly 3% between 1986 and 1989 (Table 2). Seroprevalence in *B. burgdorferi* antibody-positive subjects appeared to remain constant between 1986 and 1989.

The geographical seroprevalence of anti-*Babesia* responses was established by dividing the state into quadrants (northeast, northwest, southeast, and southwest) radiating from the city of Hartford. Samples from *Borrelia*-reactive subjects in group I and non-*Borrelia*-reactive subjects in groups IIa and IIb were analyzed. IFA reactivity was significantly greater for all subjects living in the eastern and southeastern sections of the state than for subjects living in other sections of the state (two-tailed Fisher exact test, $P < 0.01$ and $P = 0.045$, respectively) (Table 3). IFA reactivity was also greater for subjects living within 10 miles (ca. 16

TABLE 2. Reactivity against *B. microti* antigen of serum samples taken between 1959 and 1989 from residents of Connecticut

Serum sample type (group)	Yr	Samples	
		No. tested	% <i>Babesia</i> reactive
Non- <i>Borrelia</i> reactive (II [random])	1959–1963	113	0
	1964–1978	0	0
	1979–1985	23	0
	1986–1989	70	2.9
<i>Borrelia</i> reactive (I)	1986	49	10.2
	1987	349	9.2
	1988	95	9.5
	1989	242	9.9

km) of the coastline than for those living inland in the southeast section of the state (two-tailed Fisher exact test, $P = 0.055$).

All sera were tested for evidence of Lyme disease by ELISA. All group I sera had previously been determined to be reactive, and they remained reactive in our assay. Five of 304 outpatients (group II) were seropositive. These five were in group IIb and were all seronegative for *B. microti*. Only one serum sample collected in 1986 was reactive to *B. burgdorferi* in the college group (group III), and it was seronegative for *B. microti*. None of the sera from subjects having a negative history for Lyme disease or tick bite (group IV) were seropositive.

DISCUSSION

These findings suggest that about 2 to 4% of the population in some parts of Connecticut have been exposed to *Babesia microti*. The risk is even greater for those exposed to Lyme disease and for residents of the southeastern portion of the state. The prevalence of serologic reactivity to *B. microti* appears to have increased in Connecticut over the past 30 years and to have remained stable over the past 4 years, although the number of serum samples available for this aspect of the study was limited. There was no evidence of babesial infection in college students residing in Connecticut from 1959 to 1985, while 2.9% were seropositive after 1985. The first report of a cluster of six cases in Connecticut in 1989 reflects this increase in *B. microti* seropositivity (11). There was no evidence of an increase in babesial infections in Connecticut residents with Lyme disease from 1986 to 1989. The previously reported acute infections were reported in the vicinity of Mystic, located on the southeastern shore of the state, where the prevalence of Lyme disease is

greatest. Residents of other parts of the state frequently visit this resort area during the summer season of transmission, which may explain, in part, the reactivity of sera in groups II and IV.

Human infections with *B. microti* are usually asymptomatic. Clinical illness occurs primarily in elderly patients and in immunocompromised subjects, especially those who are asplenic (6–8, 12, 13, 15, 24, 25, 27). The possibility of severe *Babesia* infections in individuals with AIDS has been suggested (4). Although babesiosis is thought to be an asymptomatic infection in most immunocompetent individuals, carefully controlled studies to define the full clinical spectrum of the disease have not been carried out. Several reports of coexistent Lyme disease and babesiosis indicate that some cases of Lyme disease that are severe or prolonged may represent dual infection (12, 17). *B. microti* antibody determinations should be made in such cases; moreover, all cases of babesial infection should be tested for evidence of infection by *B. burgdorferi* (12).

The *Babesia* piroplasm is transmitted by nymphal *I. dammini* ticks, generally during the summer months (20). As many as 40% of ticks infected with *B. burgdorferi* are concurrently infected with *B. microti* in certain areas of endemicity (21). Indeed, in eastern Long Island there is intensive transmission of both *B. microti* and *B. burgdorferi*, which may account for the high rate of *Babesia* seropositivity (66%) reported for people with Lyme disease from that area (3). *I. dammini* ticks, however, generally appear to be less competent as hosts for this pathogen than as hosts for the agent of Lyme disease, which accounts for the greater prevalence of Lyme disease in areas where both agents are zoonotic (19). The Lyme disease spirochete may be passed transovarially at low frequency, and certain birds may serve as reservoir hosts (1, 28). These considerations and the efficiency with which this agent is transmitted by the tick explain the rapidly increasing range of Lyme disease (3, 30). Since *B. microti* is not inherited by the progeny of infected ticks and since birds do not serve as reservoirs, babesial infection would be expected to spread more slowly from its present areas of distribution in New England, New York, and Wisconsin than does Lyme disease (6, 7, 13, 15, 18). The intraerythrocytic location of *B. microti* facilitates efficient transmission of this agent by blood transfusion, whereas the agent of Lyme disease has only rarely been isolated from blood (9, 14). Thus, transfusion-acquired babesiosis is more frequently reported than are such cases of Lyme disease (23). Transfusion-acquired infections may help explain the prevalence of antibodies against *B. microti* in the northwest area of Connecticut, where ticks (and cases of Lyme disease) are scarce, and in those study subjects not at risk for Lyme disease (group IV).

A brisk antibody response follows babesial infection in both human and animal hosts. In one study of 16 babesiosis patients, IFA levels against babesial antigen of 1:1,024 to 1:4,096 were detected within a month after onset of illness and then declined to 1:16 to 1:256 5 to 13 months later (26). The authors concluded that patients with titers of 1:1,024 or greater had been infected recently and that those with titers of 1:64 or less had developed infection at least 6 months earlier. Although the IFA test is currently the standard assay used for detection of *B. microti* antibody in human sera, the positive cutoff value has been variously placed at levels of 1:16 and 1:64 (5, 26). One serosurvey utilizing a positive cutoff value of 1:64 demonstrated that less than 2% of randomly selected adult subjects from Cape Cod, Martha's Vineyard, and Nantucket and 8% of Nantucket subjects with

TABLE 3. Reactivity against *B. microti* antigen of serum samples from geographical quadrants of Connecticut^a

Quadrant	<i>Borrelia</i> positive		<i>Borrelia</i> negative		Total	
	No. of samples	% <i>Babesia</i> reactive	No. of samples	% <i>Babesia</i> reactive	No. of samples	% <i>Babesia</i> reactive
Northeast	33	6.1	21	4.8	54	5.6
Northwest	50	6.0	84	2.4	134	3.7
Southeast	601	10.6	121	3.3	722	9.4
Southwest	32	3.0	78	1.3	110	1.8

^a Serum samples that reacted against *B. burgdorferi* antigen (group I samples) and those that did not react against *B. burgdorferi* antigen (group II samples) were compared for *Babesia* reactivity.

a history of tick bite or fever had been exposed to the *Babesia* antigen (27). Other serosurveys in the region indicate that prevalence ranges between 3.7 and 6.9% (10, 23). Using the same cutoff value, we established a somewhat greater seroprevalence estimate for Connecticut. Any increase in prevalence may reflect the rising risk of babesial infection.

Anti-*Babesia* seroprevalence appears to have become established in mainland Connecticut during the past several decades, but it does not appear to have increased in the past several years. At present there is considerable risk of babesial infection among residents of the Connecticut mainland exposed to Lyme disease and among those living in the southeastern part of the state. Such data suggest that physicians in areas where Lyme disease and babesiosis have been reported should be aware that patients exposed to Lyme disease may also be at increased risk of babesial infection.

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REFERENCES

- Anderson, J. F., R. C. Johnson, L. A. Magnarelli, and F. W. Hyde. 1986. Involvement of birds in the epidemiology of the Lyme disease agent *Borrelia burgdorferi*. *Infect. Immun.* 51:394-396.
- 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.
- 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.
- Benezra, D., A. E. Brown, B. Polsky, J. W. M. Gold, and D. Armstrong. 1987. Babesiosis and infection with human immunodeficiency virus. *Ann. Intern. Med.* 107:944.
- Chisholm, E. S., T. K. Ruebush, A. J. Sulzer, and G. R. Healy. 1978. *Babesia microti* infection in man: evaluation of an indirect immunofluorescent antibody test. *Am. J. Trop. Med. Hyg.* 27:14-19.
- Dammin, G. J. 1978. Babesiosis, p. 169-199. *In* L. Weinstein and B. N. Fields (ed.), *Seminars in infectious disease*. Stratton Intercontinental Books, New York.
- Dammin, G. J., A. Spielman, J. I. Benach, and J. Piesman. 1981. The rising incidence of clinical *Babesia microti* infection. *Hum. Pathol.* 12:398-400.
- Desforages, J. F., and F. Quimby. 1976. Babesia abroad. *N. Engl. J. Med.* 295:103-104.
- Duray, P. H. 1989. Clinical pathologic correlations of Lyme disease. *Rev. Infect. Dis.* 11(Suppl. 6):1487-1493.
- Filstein, M. R., J. L. Benach, D. J. White, et al. 1980. Serosurvey for human babesiosis in New York. *J. Infect. Dis.* 141:518-521.
- Gadbaw, J. J., J. F. Anderson, M. L. Cartter, and J. L. Hadler. 1989. Babesiosis—Connecticut. *Morbid. Mortal. Weekly Rep.* 38:649-650.
- Golightly, L. M., L. R. Hirschorn, and P. F. Weller. 1989. Fever and headache in a splenectomized woman. *Rev. Infect. Dis.* 11:629-637.
- Healy, G. R. 1979. Babesia infections in man. *Hosp. Pract.* June:107-116.
- Johnson, R. C. 1989. Isolation techniques for spirochetes and their sensitivity to antibiotics in vitro and in vivo. *Rev. Infect. Dis.* 11(Suppl. 6):1505-1510.
- Krause, P. J. 1986. Babesiosis, p. 2019-2024. *In* R. D. Feigin and J. D. Cherry (ed.), *Textbook of pediatric infectious diseases*. The W. B. Saunders Co., Philadelphia.
- Magnarelli, L. A., J. M. Meegan, J. F. Anderson, and W. A. Chappell. 1984. Comparison of an indirect fluorescent-antibody test with an enzyme-linked immunosorbent assay for serological studies of Lyme disease. *J. Clin. Microbiol.* 20:181-184.
- 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.
- Mather, T. N., S. R. Telford, S. I. Moore, and A. Spielman. 1990. *Borrelia burgdorferi* and *Babesia microti*: efficiency of transmission from reservoirs to vector ticks (*Ixodes dammini*). *Exp. Parasitol.* 70:55-61.
- Piesman, J., J. G. Donahue, T. N. Mather, and A. Spielman. 1986. Transovarially acquired Lyme disease spirochetes (*Borrelia burgdorferi*) in field-collected larval *Ixodes dammini* (Acari: Ixodidae). *J. Med. Entomol.* 23:219.
- Piesman, J., T. N. Mather, G. J. Dammin, S. R. Telford, C. C. Lastavica, and A. Spielman. 1987. Seasonal variation in transmission risk of Lyme disease and human babesiosis. *Am. J. Epidemiol.* 126:1187-1189.
- 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.
- Piesman, J., and A. Spielman. 1982. *Babesia microti*: infectivity of parasites from ticks for hamsters and white-footed mice. *Exp. Parasitol.* 53:242-248.
- Popovsky, M. A., L. E. Lindberg, A. L. Syrek, and P. L. Page. 1987. Prevalence of babesia antibody in a selected blood donor population. *Transfusion (Philadelphia)* 28:59-61.
- Rosner, F., M. H. Zarrabi, J. L. Benach, and G. S. Habicht. 1984. Babesiosis in splenectomized adults. Review of 22 reported cases. *Am. J. Med.* 76:696-701.
- Ruebush, T. K., P. B. Cassaday, H. J. Marsh, et al. 1977. Human babesiosis on Nantucket Island. Clinical features. *Ann. Intern. Med.* 86:6-9.
- Ruebush, T. K., E. S. Chisholm, A. J. Sulzer, and G. R. Healy. 1981. Development and persistence of antibody in persons infected with *Babesia microti*. *Am. J. Trop. Med. Hyg.* 30:291-292.
- Ruebush, T. K., D. D. Juranek, E. S. Chisholm, P. C. Snow, G. R. Healy, and A. J. Sulzer. 1977. Human babesiosis on Nantucket Island. Evidence for self-limited and subclinical infections. *N. Engl. J. Med.* 297:825-827.
- Spielman, A. 1988. Lyme disease and human babesiosis: evidence incriminating vector and reservoir hosts, p. 147-165. *In* A. Sher and P. Englund (ed.), *The biology of parasitism: a molecular and immunological approach*. Alan R. Liss, Inc., New York.
- Spielman, A., M. L. Wilson, J. F. Levine, and J. Piesman. 1985. Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. *Annu. Rev. Entomol.* 30:439-460.
- Steketee, R. W., M. R. Eckman, E. C. Burgess, et al. 1985. Babesiosis in Wisconsin: a new focus of disease transmission. *JAMA* 253:2675-2678.