INCREASING HEALTH BURDEN OF HUMAN BABESIOSIS IN ENDEMIC SITES

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Abstract. Human infection due to Babesia microti has been regarded as infrequent and a condition primarily affecting the elderly or immunocompromised. To determine whether risk in endemic sites may be increasing relative to that of Borrelia burgdorferi and to define its age-related clinical spectrum, we carried out a 10-year community-based serosurvey and case finding study on Block Island, Rhode Island. Less intensive observations were conducted in nearby sites. Incidence of babesial infection on Block Island increased during the early 1990s, reaching a level about three-fourths that of borrelial infection. The sera of approximately one-tenth of Block Island residents reacted against babesial antigen, a seroprevalence similar to those on Prudence Island and in southeastern Connecticut. Although the number and duration of babesial symptoms in people older than 50 years of age approximated those in people 20 to 49 years of age, more older adults were admitted to hospital than younger adults. Few Babesia-infected children were hospitalized. Babesial incidence at endemic sites in southern New England appears to have risen during the 1990s to a level approaching that due to borreliosis.

INTRODUCTION

The history of human babesiosis is brief, dating only as far back as 1956 in Europe, 1966 in North America, and with a more recent history in Asia and Africa. 1–8 North American cases cluster mainly in the northeastern and upper midwestern United States, particularly among residents and visitors on the chain of islands in Massachusetts, Rhode Island, Connecticut, and New York that comprise the terminal moraine left by the great piedmont glaciers. 9–14 Zoonotic cycles of Babesia microti first were recognized on these islands during the 1970s and more recently on mainland sites, beginning during the 1980s. 1,13–18 Although the recognized geographic range of human babesiosis is less extensive than that of Lyme disease, recent studies suggest that it is expanding. 19–21

Far more cases of Lyme disease than human babesiosis have been noted. 12–14 This disparity in incidence can be explained in part by the fact that Lyme disease is nationally reportable in the United States while babesiosis is not. Furthermore, the erythema migrans rash that is the hallmark of Lyme disease is readily identified while patients with babesiosis generally experience a flu-like illness that is difficult to distinguish from numerous other conditions, including various viral infections. More readily diagnosed episodes of severe babesial infection seem limited to the elderly and those who are immunocompromised, particularly people lacking a spleen. 22–24 Although the incidence of human babesiosis appears to be increasing, the intensity of its transmission and the relationship of its clinical expression to age have not yet objectively been characterized. 1,11

To determine whether the incidence of babesial infection may be increasing in endemic sites and whether severe disease may be more frequent among children and young adults than is currently recognized, we conducted a 10-year prospective case-finding and serosurvey analysis of the various tick-borne zoonoses. The study was carried out in defined human populations residing in an array of sites distributed along the oceanic moraine in the northeastern United States.

MATERIALS AND METHODS

Study sites and sampling procedures. A study cohort was established by soliciting residents of Block Island, Rhode Island from 1991 through 2000 to participate in a biannual serosurvey. The study cohort was limited to residents whose sera did not react initially against antigens of B. microti or Borrelia burgdorferi and who spent at least one month during the transmission season (May through October) on the island. Block Island is particularly attractive for epidemiologic and clinical studies on tick-borne infection because it is isolated (20 km) from the New England mainland and because both infections are endemic there. It is home to approximately 1,100 year-round residents who seek health care from physicians based at the Medical Center, the sole medical facility on the island. We sought to identify all cases of infection due to the coinfesting Ixodes-transmitted pathogens B. microti, B. burgdorferi, and the agent of human granulocytic ehrlichiosis (HGE) occurring in the cohort with the help of the staff of the Block Island Medical Center and by a dedicated research nurse. 10 Infections were identified among the members of the cohort who visited the Medical Center for an acute tick-borne illness (termed passive surveillance) or who developed antibody against babesial, borrelial, or HGE antigen during the serosurvey activity (termed active surveillance). Information on disease characteristics was standardized by asking all subjects whether they had experienced a particular array of symptoms either during acute illness or at any time during the year prior to the detection of babesial, borrelial, or HGE antibodies. Medical histories were obtained and physical ex-
aminations and specific babesial, spirochetal, and ehrlichial laboratory tests were performed on all symptomatic subjects at the time of acute illness and four to six weeks later. A medical history was obtained at least every three months until the patient had become asymptomatic. Babesial-associated hospital admissions were recorded for all residents of the island. Because less than five percent of study subjects experienced HGE infection, no further analysis of these cases was carried out.

Surveillance for tick-borne infection also was conducted at three other study sites in southern New England that are highly endemic for Ixodes-borne infections. On Nantucket Island, Massachusetts, we recorded episodes of such infections among people attending two medical practices from 1991 through 2000. Nantucket is situated about 40 km from the Massachusetts mainland with approximately 3,000 year-round residents. On Prudence Island, Rhode Island, we carried out active surveillance during 1999 and 2000 with the same serosurvey methods as those used on Block Island. Prudence Island is located within Narragansett Bay and is home to approximately 300 year-round residents. Finally, in southeastern Connecticut, we carried out active surveillance for babesial antibody and recorded episodes of Ixodes-borne illness and Babesia-associated hospital admissions from 1991 through 2000. The Connecticut site is comprised of the population served by Lawrence and Memorial Hospital in New London, Connecticut, the largest hospital in southeastern Connecticut with a catchment population of approximately 130,000 people. Babesial seroprevalence in southeastern Connecticut was assessed by anonymous testing of blood donors consecutively participating in Red Cross blood drives in 2000. Blood specimens were collected in accordance with the Red Cross institutional review board. Written informed consent was obtained from all other human adult study participants and from the parents or legal guardians of minors in accordance with human experimentation guidelines approved by the institutional review boards at Connecticut Children’s Medical Center and the Harvard School of Public Health.

**Case definitions.** Diagnosis of newly acquired *B. microti* infection during the course of this study required either 1) the presence of symptoms consistent with babesiosis and laboratory evidence of recent infection or 2) seroconversion that consisted of a change from an initial nonreactive serum to a subsequent reactive serum that included antibody to *B. microti*. Babesial symptoms included fever, chills, sweats, fatigue, headache, and myalgia. Laboratory evidence of recent infection required the recognition of piroplasms by microscopic examination of a thin blood smear, as previously described.26 Laboratory evidence of recent infection or 2) seroconversion that followed an autumn or spring serosurvey and if they neither sought medical care nor reported typical symptoms consistent with babesiosis or Lyme disease during the previous year.

**Laboratory methods.** Piroplasms were identified microscopically in Giemsa-stained films of EDTA-anticoagulated blood. At least 100 fields (400x magnification) were examined before declaring the sample free of piroplasms.26 Evidence of babesial infection was assessed serologically by an indirect immunofluorescent antibody test, as previously described.27,28 For comparison, each batch of samples tested were accompanied by a serum sample taken from a patient whose blood contained babesial pathogens that were readily detected by microscopic examination of a thin blood smear, as well as another from a person who had not been exposed to babesial infection. As an additional check for the specificity of the assay, sera collected from 50 residents of Iceland in 1993, were tested and none reacted to *B. microti* or *B. burgdorferi* antigens. A reactive serum was defined as one reacting at a dilution of 1:64. Reactive specimens were tiered to their endpoint.

Serologic evidence of exposure to the Lyme disease spirochete was detected by an enzyme-linked immunosorbent assay.29 All borderline or reactive sera were further characterized by immunoblotting.29 Specimens were considered positive according to the criteria of the Centers for Disease Control and Prevention and the Association of State and Territorial Public Health Laboratory Directors.30 Whole blood samples for the PCR were analyzed and processed by personnel blinded to the clinical status of the donor. The DNA was extracted from the blood samples of singly and coinfected subjects. We targeted for amplification a 294–basepair (bp) portion of the *B. burgdorferi* outer surface protein A (Osp A) gene and a 238-bp portion of the *B. microti* nuclear small subunit ribosomal gene using previously described PCR protocols, except that the volume of blood analyzed for babesial DNA was 0.5 ml rather than 0.2 ml.31,32 The sensitivities and specificities of these assays are high (greater than 90%).31,32

**Data analysis.** Duration of exposure to tick-borne infection (person–time) was calculated for each subject in the Block Island study cohort by determining the time between the year of subject enrollment and the earliest of any of the following events: the beginning of three years of absence from the serosurvey, year of departure from Block Island, year of death, or the year 2000. Age group specific incidence rates (per 100,000 person-years of exposure) were estimated by dividing the number of cases identified among serosurvey participants of a given age group by the sum of that age group’s person-years of exposure and multiplying the result by 100,000.

Confidence intervals for incidence rate ratios comparing age groups and etiologic agents were calculated using Poisson regression models. The significance of temporal trends for babesial infections and for Lyme disease infections were assessed using Poisson regression models for the first four years.
of the study when the greatest change in babesial incidence appeared to have occurred and separately for the last six years of the study. The null hypothesis of equal temporal trends was rejected if the coefficient estimate for the interaction term was significantly different from zero.

The number and duration of babesial symptoms were estimated by selecting all confirmed symptomatic babesiosis cases identified on Block Island, Nantucket, and in southeastern Connecticut and monitoring patients until they reported no more symptoms. Lawrence and Memorial Hospital age group–specific admission rates were calculated by comparing total admissions for babesiosis with the total hospital catchment area population of the respective age groups for the period 1995 to 1998. The catchment area was determined by identifying the towns representing 95% of hospital admissions and using data from the Connecticut Department of Economic Development to estimate the 1995 population of these towns by age. Differences in the median number and duration of symptoms by age group were assessed using the Wilcoxon rank sum test. Data analysis was done by the use of Microsoft (Bellevue, WA) Excel®, JMP (SAS Institute, Cary, NC), and Stata (Stata Corporation, College Station, TX).

RESULTS

Comparison of the cohort study population and the resident population. First, we compared the number of people in our study cohort population and the duration of their exposure to tick-borne illness (person-time) with that of the entire Island population. Person-time was calculated as the difference between the initial and final visit to the serosurvey. Total person-time for all study subjects over the 10 years of the study was compared to the expected person-time if every resident of Block Island participated for the full 10 years of the study. Of the 1,487 subjects who enrolled in the study between 1991 and 2000, 1,078 spent at least six months on the island each year and were classified as full time residents according to United States Census criteria. Based upon the average of the 1990 and 2000 Census populations and the number of years each subject participated in the study, we captured nearly 60% of resident exposures (person-time) over the 10-year study period, including approximately 70% of the adult person-time and 40% of the child person-time. Thus, the majority of Block Island residents participated in our study.

Incidence of babesial infection. We next estimated the incidence of B. microti and B. burgdorferi infection and disease in our Block Island study population, including subjects who seroconverted as well as those who experienced physician-diagnosed illness. During the 10-year span of our analysis, the incidence of babesial infection was approximately three-fourths of the incidence of borrelial infection (incidence rate ratio [IRR] = 0.776, 95% confidence interval [CI] = 0.618–0.974) while the incidence of babesial disease was about one-third of that of Lyme disease (Figure 1). Children appear to have been infected about as often as adults; however, only a small number of children were studied (IRR = 1.008, 95% CI = 0.536–1.755) (Table 1). Residents of Block Island are only slightly less likely to be infected by the agent of human babesiosis than by that of Lyme disease.

We next determined whether the incidence of babesial and borrelial infection might have changed among Block Island residents during the 10-year span of observation. The number of incident babesial infections increased markedly relative to that of borrelial infections during the first four years of the 1990s (P < 0.01). The number of incident borrelial infections remained unchanged during that time (P = 0.325) (Figure 1). Subsequently, the incidence of both babesial and borrelial infections fluctuated concordantly. Risk of human babesiosis came to approximate that of Lyme disease on Block Island during the mid-1990s.

Risk of babesial infection as measured by seroprevalence was compared at diverse sites in southern New England to determine whether the incidence of babesial infection observed on Block Island might be comparable to the incidence at other babesial endemic sites. Similar seroprevalences of antibabesial antibody were noted among 223 Block Island
residents (9%) and 161 Prudence Island residents (10%) during annual serosurveys conducted between 1999 and 2000 and among 737 anonymously tested blood donors from southeastern Connecticut (9%) during the same time period. The current risk of human babesial infection at other endemic sites in southern New England may be similar to that on Block Island.

Clinical expression of babesial infection. We determined the frequency of asymptomatic babesial infection among Block Island residents. Because the interval between sampling for some study subjects exceeded one year, almost half of our subjects who experienced babesial infection could not be categorized either as asymptomatic or symptomatic. Of the remaining subjects, approximately two-thirds experienced symptomatic babesial infection, including 60% (6 of 10) of the children and 81% (54 of 67) of the adults who were infected. Children and adults who acquire babesial infection often experience symptomatic illness.

The number and duration of symptoms in 54 patients experiencing symptomatic babesial infection was described among residents of Block Island, Nantucket, and Connecticut. Younger adults (age = 20 to 49 years) had the same number and duration of symptoms as did older adults (age = 50 to 89 years), while children had fewer symptoms and did so over a shorter span of time than did adults (Table 2). The severity of babesial illness in young adults generally is similar to that in older people.

Finally, we characterized the age and immune status of those *B. microti*-infected residents of our study sites who were hospitalized. Of our 186 symptomatic babesial infected and babesial-Lyme disease coinfected subjects, 16 were hospitalized, including 13% of the 89 older adults, 5% of the 80 younger adults (the youngest was 41 years old), and none of the 17 children. Three of 16 of our hospitalized subjects were asplenic. The mean duration of hospital stay was eight days. During the study, we learned that three children from southeastern Connecticut were admitted to various hospitals with a diagnosis of babesiosis. One of the children previously had been healthy, one was a neonate, and one was asplenic. In a separate group of 52 people consecutively admitted for babesiosis to Lawrence and Memorial Hospital between 1995 and 1998, six were younger adults (the youngest was 44 years old), 46 were older adults, and all were referred from 21 surrounding towns. The average annual hospital admission rate for babesiosis per 100,000 residents of these 21 towns was 1.5 (95% CI = 0–14.5) for younger adults and 19 (95% CI = 14–25) for older adults. The town with the highest rates was Old Lyme, Connecticut with rates of 9.5 (95% CI = 0.2–53) and 132 (95% CI = 70–225), respectively. Comparable results for Block Island from 1991 to 2000 were none for younger adults and 73 (95% CI = 9–260) for older adults. While the babesial hospital admission rate for Block Island was greater than that of southeastern Connecticut, the rate in several Connecticut towns was comparable to that of Block Island. These results indicate that the incidence of babesiosis may be similar to that of Block Island in certain mainland sites. Furthermore, although severe episodes of babesiosis are most common in adults over the age of 50, babesial infection may hospitalize younger adults and children.

**DISCUSSION**

The incidence of babesial infection among the residents of our Block Island study site is at least 10-fold greater than previously recognized elsewhere. Similarly, the incidence of *Borrelia* infection there is several times greater than in earlier estimates. However, these previously reported assessments of incidence were based on less rigorous methods than the active mode of surveillance that we used. Conventional estimates of the incidence of tick-borne infection are constrained by passive modes of reporting that require the participation of practicing physicians. Our active surveillance methods permit us to detect asymptomatic and mildly symptomatic cases. Practicing physicians, of course, frequently fail to diagnose or report many such infections. Indeed, the number of Lyme disease cases in the United States may be underestimated by a factor of 12. A deterministic model used to estimate the total annual number of Lyme disease cases for Westchester County, New York arrived at an annual incidence of 1,200 cases per 100,000, a rate of symptomatic infection similar to the rate that we documented among residents of Block Island. Even if every case of tick-borne infection on Block Island occurred solely within our serosurvey cohort group and we calculated person-time exposure based upon the entire Island population, our estimates of incidence (1,291 cases per 100,000 for Lyme disease and 906 cases per 100,000 for babesiosis) would still exceed previously reported rates. Alternatively, if the estimated incidence of tick-borne infection on Block Island were based solely on symptomatic cases, our derived rates for Lyme disease and babesiosis (1,677 and 516 cases per 100,000) also would exceed those previously reported. Such estimates are inherently conservative because seroreactivity may wane during the period between tests. Thus, the risk of babesial and boorrelial infection on Block Island is intense.

Babesiosis may be acutely debilitating and mortality rates of 5% have been reported among patients with babesiosis. Many of our babesia-infected subjects experienced symptomatic infection. Even asymptomatic or mild babesial infection may be problematic. Such silent infection generally is not diagnosed or treated and may persist for months or recrudesce, thereby facilitating transmission through blood donation. Babesiosis has been regarded as a geriatric infection, which impedes the diagnosis in younger people. Although symptoms are more diverse and longer lasting in adults than in children and hospital admission rates are greater in older adults, symptomatic babesial infection and infection requiring hospital admission are not restricted to the elderly or the immunosuppressed. Physicians need to be aware that children and young adults as well as older people may experience symptomatic babesial infection and moderate to severe babesial illness.

**TABLE 2**

<table>
<thead>
<tr>
<th>Years of age</th>
<th>No. of subjects</th>
<th>No. of symptoms (mean ± SD)</th>
<th>Weeks of illness (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–19</td>
<td>5</td>
<td>5.4 ± 1.7</td>
<td>3.8 ± 4.1</td>
</tr>
<tr>
<td>20–49</td>
<td>49</td>
<td>8.4 ± 3.0*</td>
<td>7.7 ± 4.1*</td>
</tr>
<tr>
<td>50–89</td>
<td>19</td>
<td>8.8 ± 3.5</td>
<td>8.3 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>8.2 ± 2.6</td>
<td>7.5 ± 4.4</td>
</tr>
</tbody>
</table>

* 0–19 vs 20–89 years of age; *P* < 0.05.
Because babesial symptoms generally are non-specific, the diagnosis of babesial infection depends upon laboratory testing. Antibody testing is especially important in identifying asymptomatic or mild cases that do not come to the attention of physicians because parasites may not be detectable in a stained blood-smear or by a PCR, especially after acute symptoms have resolved. The antibabesial antibody assay used in this study is sensitive and specific, as demonstrated in prospective multicenter studies that compared the results of coded serum samples from well-defined cases of babesiosis and negative controls. Indeed, we find that the pattern of increase in babesial cases on Block Island is similar whether diagnosis is based on serocconversion alone or on seroconversion plus microscopic or PCR-based identification of the pathogen. Our seroprevalence results on Block Island, Prudence Island, and southeastern Connecticut are comparable to those of similar studies that were conducted elsewhere in the northeastern United States and are consistent with a relatively recent pattern of emergence of babesiosis in this region. Similarly, we found that adult hospital admission rates for babesiosis in some Connecticut communities were similar to those on Block Island. We find that the intense and apparently increasing risk of human babesial infection that characterizes an island site also may characterize certain mainland communities.

Human babesiosis in the United States first was reported during the late 1960s in residents of San Francisco, California, and Nantucket Island, Massachusetts. Endemic infestations of vector ticks were recognized in terminal moraine sites off the coast of southern New England and New York at about that time with subsequent expansion to the shoreline communities of southern New England. Zoonotic cycles of Lyme disease and babesiosis first were recognized on coastal mainland sites in the 1970s and more recently in interior mainland regions. Human babesiosis generally is detected in sites where *Ixodes* ticks are endemic only after Lyme disease has become well established. The increased incidence of babesial infection relative to Lyme disease on Block Island during the course of our study is consistent with this pattern of emergence, as is the increasing risk of babesiosis extant in southeastern coastal Connecticut and the relatively frequent Babesia-associated hospital admissions in the region. Babesial seroprevalence of residents of Prudence Island in Rhode Island and in southeastern Connecticut appear to be similar to that for Block Island. The seeds for the emergence of tick-borne disease on Block Island were sown in 1967 when seven deer were captured in the MidWest and released onto the island. Deer amplify the vector *Ixodes* ticks and their rapid increase brings with it a concomitant rise in the incidence of human tick-borne disease. However, an increase in deer does not explain the disparity between the initial appearance of Lyme disease and subsequent emergence of babesial infection. Other potential factors that might account for such a disparity include a more rapid geographic dispersion of the Lyme spirochete than the babesial parasite, greater spirochetal than babesial transmission during tick feeding, or more widespread recognition of Lyme disease than babesiosis on the part of physicians and the lay public. Physicians practicing where Lyme disease is endemic should be aware of the possible introduction and rapid emergence of human babesiosis in their region.

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REFERENCES