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Pediatrics 1992;89;1045-1048

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Babesiosis: An Underdiagnosed Disease of Children

Peter J. Krause, MD*; Sam R. Telford III, ScD§; Richard J. Pollack, PhD§; Raymond Ryan, PhD‡; Peter Brassard, MD‖; Lawrence Zemel, MD*; and Andrew Spielman, ScD§

ABSTRACT. Babesiosis is a malaria-like illness caused by the intraerythrocytic parasite *Babesia microti* and is transmitted by the same tick that transmits *Borrelia burgdorferi*, the causative agent of Lyme disease. Babesiosis is well recognized in adult residents of southern New England and New York but has been described in only five children. To determine whether children are infected with *B microti* less often than are adults, a prospective serosurvey was carried out on Block Island, RI, where babesiosis is endemic. Randomly recruited subjects completed a questionnaire and provided a blood sample. Antibodies against *B microti* and *B burgdorferi* were measured using a standard indirect immunofluorescence assay and enzyme-linked immunosorbent assay, respectively. Of 574 subjects, 9% tested positive for *B microti*, including 12% of the 52 children (7 months through 16 years) and 8% of the 522 adults (not significant, $P < .6$). Although babesiosis had not been diagnosed in any of the *Babesia*-seropositive subjects, 25% of the children and 20% of the adults reported symptoms compatible with this infection during the previous year. Of the 6 children and 45 adults seropositive for *B burgdorferi*, 17% and 14%, respectively, were also seropositive for *B microti*. It is concluded that children are infected with *B microti* no less frequently than are adults and that this infection is underdiagnosed in all age groups. Physicians who practice where Lyme disease is endemic should become familiar with the clinical presentation and diagnosis of babesiosis, both in adults and children. ***Pediatrics* 1992;89:1045–1048; *Babesiosis, Babesia microti, Lyme disease.***

ABBREVIATIONS. IFA, immunofluorescence assay; PBS, phosphate-buffered saline; NS, not significant.

Human babesiosis is a malaria-like illness caused by an intraerythrocytic zoonotic protozoan (most frequently *Babesia microti*) that is transmitted in the northeastern United States by the same deer ticks (*Ixodes dammini*) that transmit the agent of Lyme disease (*Borrelia burgdorferi*).[1–3] First described in a resident of Yugoslavia in 1957, babesiosis has appeared with increasing frequency in various parts of Europe and the United States including California, Wisconsin, New York, and southern New England.[1–4] Babesiosis appears mainly to affect older adults; of the hundreds of cases that have been described, only five involved children.[5–8] It seems paradoxical that so few children have been affected in spite of their relative intense exposure to deer ticks. Indeed, children seem to acquire Lyme disease even more frequently than do adults.

This asymmetrical age distribution of human babesiosis may derive from a tendency of *B microti* to produce asymptomatic infection in children more frequently than in adults. To examine this hypothesis, we conducted a prospective serosurvey for babesial infection in residents of Block Island, RI, a site in which the infection is endemic, and reviewed data from a large-scale retrospective babesiosis serosurvey in Connecticut. In particular, we compared serologic and parasitologic evidence of infection in these subjects with any clinical history of babesial disease.

METHODS

Prospective Serosurvey of Block Island Residents

Sera from the full-time residents of Block Island, RI, were screened for evidence of babesiosis as well as Lyme disease between September 1990 and February 1991. We invited these subjects to participate by posting notices in the local newspaper and by broadcasting over a local cable television network. After informed consent was obtained from respondents, a questionnaire was completed and a blood sample drawn. Those subjects who were seropositive for babesiosis were later interviewed, they were examined by a physician (P.J.K.), and another blood sample was obtained.

Retrospective Serosurvey of Connecticut Residents

In a previous serosurvey of 735 Connecticut residents whose sera reacted with antigen of the agent of Lyme disease between 1986 and 1989, 70 also reacted with *Babesia* antigen.[9] We contacted as many of these seroreactive subjects as possible and inquired about their age and any previous illness that might relate to babesiosis and Lyme disease. Because these blood samples generally had been obtained from various private laboratories that had mailed them to the University of Connecticut Health Center, information about many of the patients, including their age and address, was unavailable.

Immunofluorescence Assay for Antibody to *B microti*

A standard indirect immunofluorescence assay (IFA) was used to detect antibody to *B microti*.[10] 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, alternating with passage through a vector every 4 months. When at least 40% of erythrocytes were parasitized, 1 mL of blood was collected in a syringe (heparin-coated) by cardiac puncture following halothane anesthesia. The blood was centrifuged at 500 × g

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Received for publication Jul 25, 1991; accepted Sep 27, 1991.

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for 5 minutes, 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 (400 ×). A 2.5-μL drop of cell suspension was added to each well of a 12-well glass IFA slide, dried at 23°C, and stored in a desiccator at −20°C for up to 1 month. For IFA testing, slides were warmed to room temperature, test sera were diluted 1:32 in PBS, and 20 μL of the dilution was added to a slide well. Each slide was incubated for 30 minutes at 35°C, washed three times with agitation in PBS, and air dried. A 20-μL drop of fluorescein isothiocyanate-labeled goat anti-human IgG or IgM immunoglobulin (Kirkegaard and Perry, Gathersburg, MD) diluted in PBS and Evans blue (final concentration of 0.0005%) was added to each well and incubated for 30 minutes at 35°C. The slide was again washed three times with agitation in PBS, air dried, and the coverslip mounted with buffered glycerin. Slides were examined at 630×, using a 63× oil immersion objective. For comparison, each series of tests included serum from a patient with babesiosis confirmed with a blood smear (a "positive control"), serum from a normal patient (a "negative control"), and PBS. Reactions were graded on a scale of 0 to 4+. All specimens with a 1+ to 2+ reaction were retested.

A positive serum was defined as one reacting at 1:32. In an initial study of babesiosis in Connecticut residents, we adopted a conservative positive cutoff value of 1:64.[9] Using a 1:32 positive cutoff value and number-coded antibody-positive (n = 8) and antibody-negative (n = 8) test sera, we have found the sensitivity and specificity of this assay to be greater than 95%. No day-to-day variability of the test was noted. All sera were diluted to endpoint.

Enzyme-Linked Immunosorbent Assay for *B burgdorferi*

Seroreactivity to the agent of Lyme disease was determined using an assay previously described.[11] Thus, 50 μL of a standardized spirochete suspension (strain 2591, Connecticut Agricultural Experiment Station, New Haven, CT) was added to alternate wells of flat-bottom microdilution plates (NUNC Immunoplate, Marsh, Rochester, NY). In the other wells, 50 μL of PBS was added as a control for nonspecific binding. Plates were dried overnight at 37°C, then wrapped and stored at −20°C. At the time of testing, 200 μL of PBS containing 5% horse serum and 0.01% dextran sulfate was added to each well and incubated at 37°C for 1 hour to block unused protein binding sites. Plates were washed six times with PBS-Tween 20, and 60 μL of patient sera diluted 1:160 and 1:320 was added to different wells. Plates were then incubated for 1 hour at 37°C in a humid chamber, then washed six times with PBS-Tween 20 followed by the addition of 60 μL of peroxidase-labeled anti-human IgG (1:5000) or anti-human IgM (1:5000). Following an incubation for 1 hour in a 37°C humid chamber, the plates were washed again 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 IgG or 0.5 for IgM. A sample was considered positive when the net absorbance (antigen well minus the nonspecific binding well) was three standard deviations or more above the mean absorbance of the negative control wells.

Babesia Detection in Human Peripheral Blood

Thin blood smears were prepared from ethylenediaminetetraacetate-anticoagulated blood samples, fixed in absolute methanol, and stained for 1 hour in 5% Giemsa at pH 7.0. The slides were washed, dried, and coverslipped. Each was viewed microscopically at 400× magnifications using a high-dry objective. At least 100 such fields were examined before declaring the sample free of piroplasms. When objects suggestive of piroplasms were seen, diagnosis was established by means of an oil-immersion objective providing 1000× magnifications.

Hamster Inoculation to Detect *B microti*

Female golden hamsters (*Mesocricetus auratus*) weighing 50 to 75 g were intraperitoneally inoculated with 1 mL of ethylenediaminetetraacetate-anticoagulated blood. Duplicate hamsters were monitored by thin blood films taken from blood welling from a cut on the tip of the tail and were examined as described. Fresh smears were performed every 10 days for 3 months.

Statistical Analyses

Differences among groups were examined with a χ^2 analysis or *t* test as appropriate. A *P* value of <.05 was considered significant.

RESULTS

First, we described the age and sex distribution of Block Island residents whose sera reacted against *B microti* antigen. Blood samples were obtained from 72% of the 800 permanent island residents, including 52 children (median 10 years, range 7 months through 16 years) and 522 adults (median 48 years, range 17 through 83 years). Of the 52 children, 29 (56%) were male and 23 (44%) female while of the 522 adults, 260 (50%) were male and 262 (50%) were female. The sera of 9% of these 574 subjects contained IgG antibody that reacted with *B microti* antigen; none contained reactive IgM antibody (Table 1). Sera of 6 (12%) of the 52 children contained reactive antibody, a prevalence estimate that does not differ from that of adults (not significant [NS], $\chi^2 = 0.25$, $P < .6$). The geometric mean titer was greater in children (144 ± 5) than in adults (86 ± 3, NS, $P < .3$). The percentage of males whose sera contained antibody that reacted with *B microti* antigen (14% in children and 9% in adults) was not significantly different than that of females (9% in children and 8% in adults; NS, $\chi^2 = 0.3$ and 0.1, respectively, $P < .6$ and <.8, respectively). The potential effect of bias in this body of data is minimized because our study sample included all but about one quarter of the total population of the island. We concluded that Block Island children were exposed to babesial infection about as frequently as were adults.

We recorded the prevalence of antibabesial antibody in a sample of residents of Connecticut whose sera reacted with the agent of Lyme disease. In all, the sera of 735 people were studied. Information on age was available from 227 of these subjects. The sera of 46 of these 227 subjects reacted with *B microti* antigen. As in the Block Island study, the proportion of apparently infected children did not differ from that of adults (Table 1).

The age distribution of Block Island subjects seropositive for *B microti* was then analyzed in approximately 20-year increments (17 through 39, 40 through 59, 60 through 83 years) (Figure). Although the proportion of seropositive children may have marginally exceeded that of adults in any of the age divisions analyzed, no differences between age increments could be demonstrated. A similar age distribution of seropositivity was evident when *B burgdorferi* antigen was used. In both groups, children were as likely to be seropositive as were adults. Overall, the sera of about 9% of Block Island subjects reacted with *B microti* antigen.

None of the blood smears obtained from subjects seropositive for *B microti* contained piroplasms. Viable *B microti* organisms were detected in hamsters injected with blood from 1 of 23 seropositive adults and from none of 3 seropositive children. The adult whose

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TABLE 1. Prevalence of Antibody Reacting With *Babesia microti* Antigen in Sera of Subjects Sampled on Block Island (RI) and Selected Areas of Connecticut*

Location	Children		Adults		Total	
	No.	% Pos	No.	% Pos	No.	% Pos
Block Island	52	12	522	8	574	9
Connecticut	57	16	170	22	227	21

* Comparison of data within the Block Island and Connecticut groups indicated that the sera of children reacted with *B microti* antigen about as frequently as did those of adults. Connecticut results could not be compared with those from Block Island because of differences in sampling technique. More Connecticut subjects whose sera were reactive against *B microti* antigen (46 of 74) were contacted than were nonreactive subjects (181 of 661). Although an antibody titer of 1:32 against *Babesia* antigen was found to correlate with babesial infection in the present study (see text), a titer of 1:64 was used as the cutoff for seropositive subjects in a previous publication.[9]

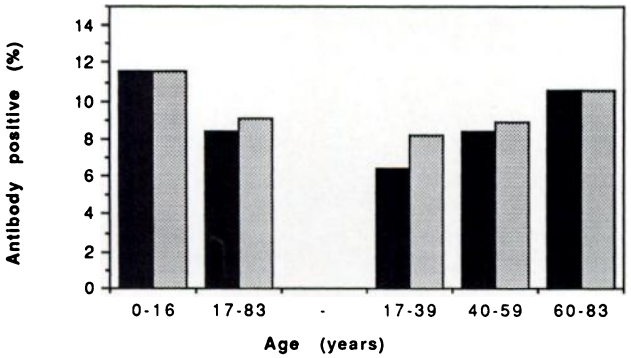

Figure. Age distribution of Block Island residents whose sera reacted with *Babesia microti* antigen (dark bars) and those whose sera reacted with *Borrelia burgdorferi* antigen (stippled bars).

blood infected hamsters was a 64-year-old woman with a *B microti* antibody titer of 1:1024. Although this finding of an active infection supports the validity of our serologic test, seropositivity generally appears to reflect a remote episode of past infection.

We then considered the hypothesis that babesial infections are underdiagnosed in children because pediatric infections may be less symptomatic than those of adults. We compared the percentage of children and adults seropositive for *B microti* who had experienced characteristic babesial symptoms. Subjects were considered to have had an illness consistent with babesiosis if they were ill for more than 1 week and had fever and chills and one or more of the following: fatigue, diaphoresis, myalgia, arthralgia, or evidence of hemolysis. Subjects with a history of Lyme disease were excluded from the analysis because any babesia-related symptoms may actually have been due to Lyme disease. Although babesiosis had not been diagnosed in any of the seropositive subjects, 1 (33%) of 3 children and 5 (15%) of 34 adults reported an illness compatible with babesiosis during the previous year. We did not ask seronegative subjects whether they may have experienced similar episodes of illness. On physical examination, none of the 6 seropositive children or 45 seropositive adults had splenomegaly or other pathognomonic signs of babesiosis. These observations suggest that babesiosis generally produces an episode of mild disease in children but do not permit rigorous correlation of severity with age.

DISCUSSION

We have demonstrated that *B microti* infection is at least as prevalent in children residing where this zoonosis is intense as in adults. Although Lyme disease is more prevalent, the age distribution of the agents is similar. This commonality is consistent with the mode of transmission of these zoonoses. Both cycle between white-footed mice (*Peromyscus leucopus*) and subadult deer ticks, and both kinds of host frequently are coinfected. The paucity of cases reported in children, thus, does not appear to result from a lesser level of infection in children than in adults. The proportion of *B microti*-infected adult residents of the nearby island of Nantucket who express relevant symptoms (30%) is similar to that observed in the present study.[12] These considerations indicate that babesial infection is underreported in children, but not because pediatric infection is nonexistent or unapparent.

Although babesial infection in children is not silent, the intensity of the illness in adult hosts appears to be greater in those older than 40 years of age than in younger adults.[12] Interestingly, the published reports of pediatric babesiosis assembled in Table 2 indicate that this condition may result in debilitating episodes of disease.[5-8] Additional work is needed to clarify the clinical spectrum of babesial illness in children as well as adults. The scarcity of reported pediatric diagnosis of babesiosis may derive from the general concept that babesiosis is a geriatric rather than a pediatric disease. Indeed, a greater number of febrile illnesses affect children than adults, and any febrile illness in an adult tends to be more aggressively evaluated than a similar problem in a child. Our study indicates that babesiosis is underreported in all age groups. Practicing physicians commonly fail to consider a diagnosis of babesiosis.

Diagnosis of babesiosis depends on recognition of an array of clinical signs, including fever, chills, fatigue, splenomegaly, and anemia. Alone and in combination they resemble those of a variety of more prevalent infections.[1-3] Specific diagnosis can be rendered by (1) direct observation of stained thin-films prepared from patient's blood, (2) amplification of infection by injecting patient's blood into hamsters, or (3) observing a fourfold rise in antibody titer. Babesiosis should be considered in any resident of an enzoonotic region who becomes febrile without an obvious cause. A reported bite by a small tick or of

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TABLE 2. Clinical Summary of Previously Reported Pediatric Cases of Babesiosis due to *Babesia microti*

Age	Illness Severity	Illness Duration, d	Erythrocytes Infected, %	Ab Titer	Antibiotic*	No. of Transfusions	Reference
2 mo	Severe	3	8	64	chloro quin clinda	3	5
14 y	Moderate	4	2	1024	quin clinda	0	6
3 wk	Moderate	5	6	1024	quin clinda gent	1	7
2 mo	Mild	2	1	0	amp gent clinda	0	7
1 mo	Moderate	5	5	512	amp gent quin clinda	1	8

* chloro, chloroquine; quin, quinine; clinda, clindamicin; amp, ampicillin; gent, gentamicin.

infection by the agent of Lyme disease should alert medical practitioners to consider babesiosis in the differential diagnosis, regardless of age of the patient. Although babesiosis became a reportable disease in Rhode Island in 1989, no indigenous cases have yet been reported in spite of our evidence demonstrating infection in at least 50 Block Island residents. The infection is widespread, affecting residents of Connecticut as well as other parts of the region.

CONCLUSIONS

Physicians who practice where Lyme disease is known to be endemic should be alert to the possibility of babesial infection in any febrile patient, particularly in those suffering from Lyme disease. Nymphal *I dammini* commonly carry both organisms, and complications resulting from dual infection may be more severe than from either infection alone. Because babesial infection in children is not always silent, the index of suspicion should extend to pediatric practices. We believe that the dearth of reports of clinical babesiosis in children may result from a masking effect due to other infections.

ACKNOWLEDGMENTS

We are indebted to the following for their help with this study: Norman and Dorothy Dahl, Linda Closter, Betty Fitzpatrick, Nancy Greenaway, Peter Wood, Ann Beth Fitterman, Feliciano Dias, Irene Kwasnik, Paula Lempart, Georgine Burke, and Trudy Lerer.

REFERENCES

1. Dammin GJ. Babesiosis. In: Weinstein L, Fields BN, eds. *Seminars in Infectious Disease.* New York, NY: Stratton Intercontinental Books; 1978:169–199
2. Healy GR. Babesia infections in man. *Hosp Pract.* 1979;June:107–116
3. Krause PJ. Babesiosis. In: Feigin RD, Cherry JD, eds. *Textbook of Pediatric Infectious Diseases.* Philadelphia, PA: WB Saunders; 1986:2019–2024
4. Dammin GJ, Spielman A, Benach JI, Piesman J. The rising incidence of clinical *Babesia microti* infection. *Hum Pathol.* 1981;12:398–400
5. Wittner M, Rowin KS, Tanowitz HB, et al. Successful chemotherapy of transfusion babesiosis. *Ann Intern Med.* 1982;96:601–604
6. Mathewson HO, Anderson AE, Hazard GW. Self-limited babesiosis in a splenectomized child. *Pediatr Infect Dis.* 1984;3:148–149
7. Scimeca PG, Weinblatt ME, Schonfeld G, et al. Babesiosis in two infants from Eastern Long Island, NY. *AJDC.* 1986;140:971
8. Esernio-Jenssen D, Sciemca PG, Benach JL, et al. Transplacental/perinatal babesiosis. *J Pediatr.* 1987;110:570–572
9. Krause PJ, Telford SR, Ryan R, et al. Geographical and temporal distribution of babesial infection in Connecticut. *J Clin Microbiol.* 1991;29:1–4
10. Chisholm ES, Ruebush TK, Sulzer AJ, Healy GR. *Babesia microti* infection in man: evaluation of an indirect immunofluorescent antibody test. *Am J Trop Med Hyg.* 1978;27:14–19
11. Magnarelli LA, Meegan JM, Anderson JF, Chappell WA. Comparison of an indirect fluorescent-antibody test with an enzyme-linked immunosorbent assay for serological studies of Lyme disease. *J Clin Microbiol.* 1984;20:181–184
12. Ruebush TK, Cassaday PB, Marsh HJ, et al. Human babesiosis on Nantucket Island. *Ann Intern Med.* 1977;86:6–9

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