PERSISTENT PARASITEMIA AFTER ACUTE BABESIOSIS

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ABSTRACT

Background   Babesiosis, a zoonosis caused by the protozoan *Babesia microti,* is usually not treated when the symptoms are mild, because the parasitemia appears to be transient. However, the microscopical methods used to diagnose this infection are insensitive, and few infected people have been followed longitudinally. We compared the duration of parasitemia in people who had received specific antibabesial therapy with that in silently infected people who had not received specific therapy.

Methods   Forty-six babesia-infected subjects were identified from 1991 through 1996 in a prospective, community-based study designed to detect episodes of illness and of seroconversion among the residents of southeastern Connecticut and Block Island, Rhode Island. Subjects with acute babesial illness were monitored every 3 months for up to 27 months by means of thin blood smears, *Bab. microti* polymerase-chain-reaction assays, serologic tests, and questionnaires.

Results   Babesial DNA persisted in the blood for a mean of 82 days in 24 infected subjects without specific symptoms who received no specific therapy. Babesial DNA persisted for 16 days in 22 acutely ill subjects who received clindamycin and quinine therapy (P=0.03), of whom 9 had side effects from the treatment. Among the subjects who did not receive specific therapy, symptoms of babesiosis persisted for a mean of 114 days in five subjects with babesial DNA present for 3 or more months and for only 15 days in seven others in whom the DNA was detectable for less than 3 months (P<0.05); one subject had recrudescent disease after two years.

Conclusions   When left untreated, silent babesial infection may persist for months or even years. Although treatment with clindamycin and quinine reduces the duration of parasitemia, infection may still persist and recrudesce and side effects are common. Improved treatments are needed. (N Engl J Med 1998;339:160-5.)

T HE clinical spectrum of human babesiosis ranges from an apparently silent infection to a fulminant malaria-like disease resulting in severe hemolysis and occasionally in death.[1-3] In eastern North America, this emerging zoonotic disease is caused by a rodent-borne piroplasm, *Babesia microti,* and is transmitted by the same ixodid tick that transmits the agents of Lyme disease and human granulocytic ehrlichiosis.

Although the natural reservoir of *Bab. microti,* the white-footed mouse (*Peromyscus leucopus*), remains parasitemic for life, human hosts appear to be infected only transiently. Limited information based on case reports suggests that microscopically demonstrable parasitemia disappears within two months in untreated subjects and within a week or two when clindamycin and quinine have been administered.[4,5] Immunocompromised subjects, however, appear to sustain infection more chronically.[6-9] The frequency of persistent parasitemia due to such blood-borne protozoan pathogens as *Bab. bigemina, Theileria parva,* and *Plasmodium vivax,* and the difficulty of detecting sparse babesial parasitemia, suggest that persistent babesial infection may occur more commonly than supposed.[10-12] Such chronic infection may increasingly threaten humans, because the pathogen can be transmitted in transfused blood and because the disease may recur.[5,6,8,13-16]

Systematic longitudinal analysis with a diagnostic test that sensitively detects babesial parasites may help determine whether a patient has persistent parasitemia.[17,18] We used the polymerase chain reaction (PCR) to compare the persistence of *Bab. microti* DNA in the blood of people who had received antibabesial drugs with that in people who had received no such drugs.

METHODS

Study Subjects

The duration of *Bab. microti* illness and of parasitemia was prospectively analyzed among residents of southeastern Connecticut and Block Island, Rhode Island, between 1991 and 1996. The staff of the sole medical center on Block Island sought to identify all episodes of Lyme disease or babesiosis that occurred there throughout the study. Subjects in Connecticut were recruited from the Pequot Clinic in Groton and from nearby private medical practices. In order to enroll the greatest possible number of infected subjects, people with symptoms suggestive of babesiosis (including malaria-like and flulike illnesses),

Lyme disease, or ehrlichiosis were included, because these infections tend to be transmitted together.

The subjects were interviewed and underwent a physical examination. Laboratory studies included examination of Giemsa-stained thin blood smears, attempted amplification of *Bab. microti* and *Borrelia burgdorferi* DNA, and evaluation of serologic reactivity against *Bab. microti, Borr. burgdorferi,* and *Ehrlichia equi* antigens. Babesial infection was diagnosed if the *Bab. microti* antibody titer increased by a factor of four or more in serum samples obtained during both the acute and convalescent phases, and if characteristic *Bab. microti* parasites were detected in thin blood smears or circulating *Bab. microti* DNA was detected by PCR. Lyme disease was diagnosed if a characteristic erythema migrans rash[19] developed, with or without an increase in the *Borr. burgdorferi* antibody titer by a factor of four or more in serum samples obtained during both the acute and convalescent phases, or if circulating *Borr. burgdorferi* DNA was amplified by PCR.

A second group of babesia-infected subjects was identified through a yearly community-based serologic survey of 1156 of the 1200 long-term residents of Block Island who were there for at least one month during the Lyme disease–transmission season between 1990 and 1996. In the autumn or spring of each year, participants were asked to provide a medical history and submit a sample of blood for *Bab. microti* PCR analysis and tests for antibodies to *Bab. microti, Borr. burgdorferi,* and the agent of human granulocytic ehrlichiosis. Babesial infection was diagnosed in a subject if both *Bab. microti* DNA and *Bab. microti* antibody were detected and if both had been undetectable the previous year.

Determination of the Duration of Parasitemia

To measure the persistence of parasitemia, blood was actively sampled in subjects who volunteered for our longitudinal serologic surveys and from others who appeared at one of our participating clinics. Samples were taken and a symptom questionnaire completed during the acute phase of their infection, 1 and 3 months later, and every 3 to 6 months thereafter for 6 to 27 months. All subjects were tested until the blood smear and PCR assay were negative. A subject was considered to have parasitemia if *Bab. microti* parasites could be detected microscopically in a Giemsa-stained thin blood smear or if *Bab. microti* DNA could be amplified by PCR.

Preparation of Blood Smears

Piroplasms were diagnosed microscopically in Giemsa-stained films prepared from EDTA-anticoagulated blood.[3] At least 100 fields were examined at a magnification of 400 before the sample was declared free of piroplasms. Objects suggestive of piroplasms were further scrutinized at a magnification of 1000.

Assays for Antibabesial Antibody

Babesial infection was diagnosed serologically by an indirect immunofluorescence assay.[20] Slides were examined at a magnification of 630 under epifluorescence. For comparison, each series of tests included serum from a subject with babesiosis (positive control), serum from a healthy adult (negative control), and phosphate-buffered saline. A positive specimen was defined as one that reacted at a dilution of 1:32 or greater.

Assays for Antispirochetal Antibody

Serologic evidence of exposure to Lyme disease spirochetes initially was detected by an enzyme-linked immunosorbent assay (ELISA).[21] A standardized spirochete suspension (strain 2591, Connecticut Agricultural Experiment Station, New Haven) was added to the wells of flat-bottomed microdilution plates (Nunc Immunoplate, Marsh, Rochester, N.Y.) alternating with wells containing phosphate-buffered saline to test for nonspecific binding. A sample was considered reactive if the net absorbance (the value for the antigen well minus the value for the nonspecific-binding well) exceeded that of the mean absorbency of the wells containing phosphate-buffered saline by 3 SD or more. Evidence of infection was provided by a serum that reacted with antigen at a dilution equal to or greater than 1:320 for IgG and greater than 1:160 for IgM.

All reactive (or borderline) serum samples were further characterized by immunoblotting against sonicated *Borr. burgdorferi* 2591.[22] The molecular masses of the reactive bands were determined by comparing their mobility with that of transblotted, prestained molecular-mass markers (Bio-Rad Laboratories, Hercules, Calif.) and with that of bands recognized by a well-characterized serum sample. Specimens were considered positive if the IgG immunoblot contained 5 or more of the 10 most common *Borr. burgdorferi*–specific bands or if the IgM immunoblot contained 2 of the following 3 bands: 23, 39, and 41 kd.[22]

PCR Assay for Piroplasm DNA

One of the investigators, who was unaware of the clinical status of the study subjects, conducted all *Bab. microti* PCR amplifications.[17] A 238-bp portion of the *Bab. microti* nuclear small-subunit ribosomal gene was targeted for amplification with a PCR protocol described previously, except that the volume of blood analyzed was 0.5 ml rather than 0.2 ml.[17]

Statistical Analysis

The chi-square test, Fisher's exact test with two-tailed comparisons, and Student's t-test were used to analyze differences among patient groups in the persistence of parasitemia and symptoms. A P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Severity of Quinine-Associated Drug Reactions

In about one quarter of our sample of 46 babesia-infected subjects, the infection was detected in the course of a serologic survey, and the subjects remained asymptomatic or had such mild symptoms that no treatment was administered (Table 1). In another quarter, the infection was detected in the course of a survey of patients two to four weeks after the diagnosis of Lyme disease. No specific antibabesial therapy was administered, because their general health had already begun to improve as a result of specific anti–Lyme disease therapy. One patient in this group did not have Lyme disease but was not treated for babesiosis because she had mild illness. Subjects who received no specific antibabesial therapy, such as clindamycin and quinine, were considered to have been untreated. The remaining half of our subjects had acute babesial illness and subsequently received specific therapy consisting of 600 mg of clindamycin and 650 mg of quinine every eight hours for one to two weeks. Three of these treated subjects were asplenic. No other subjects were asplenic or appeared to be immunosuppressed. Sex did not appear to be correlated with the array of symptoms. Patients who were sufficiently ill to require treatment were generally older than those who remained asymptomatic (Table 1), and they tended to be asplenic.

We then differentiated symptoms attributable to babesiosis from those that were more consistent with reactions to quinine. All but three of the treat-

TABLE 1. CHARACTERISTICS OF *BABESIA MICROTI*–INFECTED STUDY SUBJECTS.

CLINICAL STATUS	NO.	QUININE TREATMENT	MEDIAN AGE (RANGE)	SYMPTOMS SUGGESTING BABESIOSIS	SYMPTOMS SUGGESTING QUININE TOXICITY*	MEAN (±SD) DURATION OF SYMPTOMS
			yr	percent		days
Asymptomatic	12	No	47 (4–81)	0	0	—
Symptomatic, untreated	12	No	45 (20–68)	100	0	57±102
Symptomatic, treated	22	Yes	66 (10–87)	100	41	41±33

*The symptoms of quinine toxicity included impaired hearing (three subjects), tinnitus (one), acute episodes of hypotension during intravenous infusion (two), and gastrointestinal symptoms (three).

ed subjects required hospitalization. Of the treated subjects, almost half had symptoms that were consistent with reactions to quinine, including hearing loss, tinnitus, hypotension, and such gastrointestinal symptoms as anorexia, vomiting, and diarrhea (Table 1). In four subjects, these apparent drug reactions were sufficiently severe that therapy was discontinued after one to eight days. In another two subjects, the attending physician opted to reduce the dose of quinine. Thus, the quinine-containing regimen generally used to treat human babesiosis appeared to produce illness in nearly half the patients.

Persistence of Babesial DNA after Treatment

The persistence of circulating *Bab. microti* DNA in untreated subjects was compared with that in treated subjects. Because babesial DNA circulated about as long in untreated subjects identified through case finding as in those identified through the serologic survey and as long in partially treated as in fully treated subjects, data on all such groups were pooled in subsequent analyses. Although parasites were initially detected microscopically in the blood of two of the untreated subjects and all the treated subjects, none could be found a week after the onset of illness. Babesial DNA persisted for a mean of 82 days in subjects who received no specific therapy and 16 days in those who received therapy (P=0.03). *Bab. microti* DNA persisted for more than one month in 54 percent of the 24 untreated subjects and 36 percent of the 22 treated subjects (P=0.4) and for more than three months in 25 percent of the untreated subjects and none of the treated subjects (P=0.02). These results were determined with the assumption that parasitemia persisted to the date of the last positive PCR test. Because parasitemia may have persisted beyond the last time that babesial DNA could be amplified, an alternative test was based on the assumption that DNA persisted to the date of the first negative PCR test. This test also showed that DNA circulated longer in untreated than in treated subjects (Fig. 1). Thus, although *Bab. mi-*

croti DNA often remains in the blood for more than a month, specific antibabesial therapy reduces its persistence.

The experience of one initially asymptomatic subject is instructive. *Bab. microti* DNA was amplified from his blood during the 5th and 17th months after parasites were first detected. He then had his first apparent episode of babesial illness and was hospitalized with 3 percent parasitemia. He was febrile and had rigors, sweats, anorexia, nausea, and stupor. During hospitalization, a primary intracapsular renal tumor was identified. After a standard one-week course of clindamycin and quinine therapy, he became asymptomatic and microscopically detectable parasites disappeared from his blood. Parasites were discovered once again, however, in about 1 percent of his erythrocytes 6 weeks later (27 months after the initial parasitemia), when the affected kidney had been scheduled for removal. Therapy with clindamycin and quinine was reinstituted for another week. One week, three months, and one year after surgery, neither microscopy nor DNA amplification revealed babesial parasites. This experience indicates that babesial infection may recrudesce after many months of asymptomatic parasitemia and that, although a standard course of clindamycin and quinine therapy usually is effective, it may fail.

Coinfection and Persistence of DNA

We determined whether the presence of coinfecting Lyme disease spirochetes prolonged the duration of detectable babesial DNA. A subject was considered to be coinfected if our previously described criteria for acute babesiosis and the criteria of the Centers for Disease Control and Prevention for Lyme disease were satisfied. Of the 16 of our 46 subjects who appeared to be coinfected, only 2 received specific antibabesial therapy. Among these treated subjects, babesial DNA persisted for a mean of 17 days in the 2 spirochete-reactive subjects and 16 days in the 20 subjects who appeared not to have been exposed to the agent of Lyme disease. Parasitemia
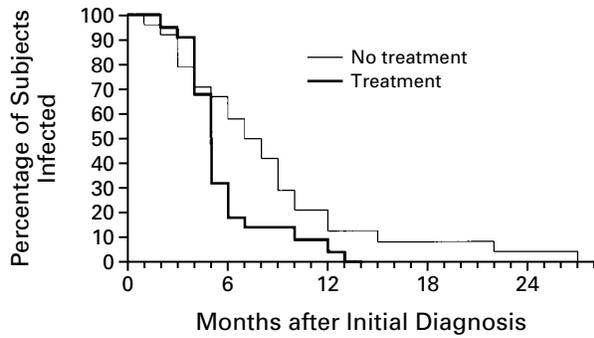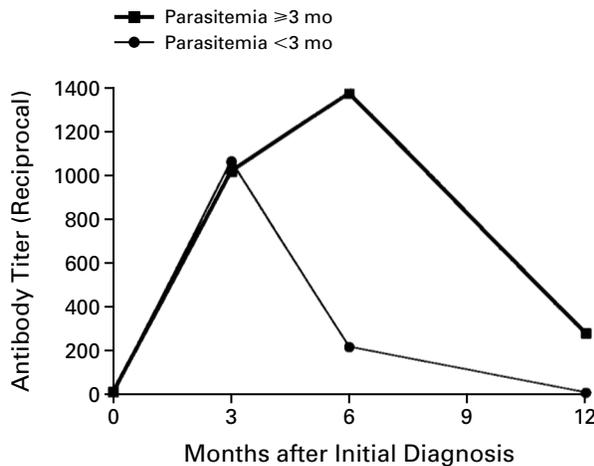
Figure 1. Persistence of DNA Amplifiable by PCR in the Blood of *Babesia microti*–Infected Subjects Who Received Specific Antibabesial Therapy as Compared with That in the Blood of Subjects Who Received No Such Treatment.

The 22 subjects treated with clindamycin and quinine had a shorter duration of parasitemia than the 24 untreated subjects (P=0.05 by the log-rank test).

No. Tested

≥3 mo	5	4	3	2
<3 mo	7	6	3	3

Figure 2. Persistence of DNA Amplifiable by PCR in the Blood of *Babesia microti*–Infected Subjects, as Compared with the Persistence of Antibabesial IgG Antibody in Their Blood.

Five subjects in whom DNA persisted for three or more months are compared with seven subjects in whom DNA persisted for less than three months.

in untreated subjects persisted for a mean of 2.4 months in the 14 spirochete-reactive subjects and 3.0 months in the 10 nonreactive subjects. The serum of one subject reacted against the agent of human granulocytic ehrlichiosis as well as against that of Lyme disease; his parasitemia persisted for 208 days. Thus, coinfecting Lyme disease spirochetes do not appear to prolong the persistence of babesial parasitemia.

Symptoms, Antibabesial Seroreactivity, and Persistence of DNA

The persistence of circulating babesial DNA was compared with the duration of symptoms and antibabesial seroreactivity in the 12 untreated subjects whose infection was detected incidentally because they had had babesial illness but had received medication without antibabesial activity. All but one were treated for 10 to 31 days with amoxicillin or doxycycline. None were treated with clindamycin and quinine, because the diagnosis of babesiosis was delayed and patients had improved by the time that the etiology was established. In the five subjects in whom babesial DNA remained blood-borne for 3 or more months, the symptoms lasted for more than 3 months (a mean of 114 days), whereas in the seven subjects in whom babesial DNA persisted for less than 3 months, the symptoms lasted only half a month (a mean of 15 days; P<0.05). Thus, persistent symptoms of babesiosis accompanied persistent blood-borne babesial DNA.

We next compared the persistence of circulating babesial DNA with that of seroreactivity against babesial antigen. IgG antibody concentrations were compared in seven subjects who retained circulating DNA for less than three months and in five subjects who retained circulating DNA for three or more months. The mean reciprocal immunofluorescence titers for the two groups were identical during the acute phase of the illness and were similar three months later. Subsequently, however, the immunofluorescence titers began to decline, so that the persistence of seroreactivity increasingly correlated with the persistence of babesial DNA (Fig. 2). Thus, antibabesial seroreactivity appears to persist longer when babesial DNA can be detected.

DISCUSSION

These observations suggest that asymptomatic human babesial infection may persist for months or even years. Even after clindamycin and quinine have been administered, infection may continue for more than two months. Silent infection, of course, may recrudesce spontaneously or after splenectomy or immunosuppression. Our findings are consistent with findings on the persistence of babesial infection in animals. *Bab. gibsoni*–infected dogs, for example, may carry chronic infection even after seemingly successful therapy, and some have signs of chronic disease, including liver lesions and chronic membranoproliferative glomerulonephritis.[23] *Bab. microti* infection in hamsters may last two or more years, with rising levels of parasitemia accompanied by ascites, anorexia, and lethargy during the last month of life.[3] We therefore suggest that human babesiosis may be more persistent and less benign than previously thought.

The presence of detectable babesial DNA in a pa-

tient's blood correlates with the persistence of symptoms and of seroreactivity and suggests the persistence of a viable infection. In general, microbial DNA is rapidly cleared from the blood, presumably by nuclease activity that is present in tissue and body fluids.[24-26] The possibility that free DNA or nonviable babesia persists in human blood for many weeks therefore seems unlikely. Our ability to amplify babesial DNA after parasitemia could no longer be detected microscopically may be due to the superior sensitivity of the PCR assay. *Bab. microti* is more readily demonstrable in human blood by PCR than by microscopical examination of thin blood smears.[18] The PCR is also more sensitive than microscopy for detecting chronic *Bab. bovis* or *Bab. bigemina* infection in cattle. The density of amplifiable DNA correlates closely with microbial density in infection by *Plas. vivax, Borr. burgdorferi, Chlamydia trachomatis,* or herpes simplex virus.[25-28] Our study of human babesiosis confirms other studies showing that microbial DNA is rapidly cleared from the blood after successful treatment of infection. Circulating *Bab. microti* DNA therefore appears to mark active parasitemia.

Specific antibabesial therapy is generally withheld from people with only mild symptoms, because such infections appear benign and because the standard course of therapy may have toxic effects.[2,4,6] It seems evident, however, that persistent, silent babesial parasitemia may have various adverse outcomes. Our subjects whose parasitemia lasted three or more months had prolonged symptoms. Silent infections, which occur in about a third of infected people,[4] may recrudesce. One of our study patients had an apparent recrudescence of babesiosis 26 months after the initial infection. Although he may have had reinfection rather than recrudescence, this episode occurred during April, when the risk of human infection is nil and when he may have been immunosuppressed as a result of cancer.

Silently infected persons also present some risk to others who may receive their transfused blood. Indeed, transfusion-transmitted babesiosis has been reported repeatedly, and the frequency of this condition is likely to rise as the zoonosis continues to emerge.[2,3,5-8,13-16] Prompt elimination of mildly symptomatic as well as silent babesial infection would protect both the infected person and transfusion recipients. Because a course of clindamycin and quinine reduces the duration of parasitemia, such a regimen should be considered for any babesia-infected person, regardless of symptoms.

Systematic treatment of those with silent babesial infection requires improved diagnostic procedures. In persons with severe symptoms of babesiosis, such as intense fever, chills, extreme fatigue, and severe anemia, the disease is usually correctly diagnosed and promptly treated, but in those with only subtle symptoms, it often remains undiagnosed. Furthermore, physicians tend not to recognize babesial infection in patients who are coinfected with the agent of Lyme disease, because babesial symptoms tend to be ascribed to Lyme disease.[29] A *Bab. microti*–specific ELISA would be a useful replacement for the current fluorescent antibody procedures for detecting antibody. Similarly, a rapid diagnostic test for antigen would replace microscopy and PCR. At present, however, physicians practicing in areas where this infection is zoonotic should be alert to the possibility of babesiosis in patients with flulike systemic symptoms during the summer and should consider using microscopy, *Bab. microti* PCR assays, and IgM immunofluorescence antibody testing for diagnosis.[17,18,30]

Improved therapeutic regimens for human babesiosis would also be useful. Clindamycin and quinine, which make up the currently accepted treatment regimen, frequently produce adverse reactions, mainly tinnitus and abdominal distress. About a fifth of treated subjects fail to complete their prescribed seven-day regimen. Recent observations, however, suggest that therapy with a combination of atovaquone and azithromycin may cure human babesiosis.[31,32] This experimental regimen appears to be relatively free of side effects. This emerging infection requires attention, because human babesial infection may persist silently for many months and because this apparently benign condition may recrudesce.

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