# Seroprevalence and Seroconversion for Tick-Borne Diseases in a High-Risk Population in the Northeast United States

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**PURPOSE:** To determine the prevalence of serologic reactivity, the 1-year incidence of seroconversion, and the frequency of multiple infections, and their associations with symptoms in a group of volunteers at high risk for tick-borne infections in New York state.

**METHODS:** We performed a seroepidemiologic study of Lyme borreliosis, 2 of the ehrlichioses, Rocky Mountain spotted fever, and babesiosis among 671 participants who lived or worked in a high-risk area (mainly in eastern Long Island, New York) for tick-borne diseases. Sera were collected in the winters of 1994 and 1995. Signs and symptoms of tick-borne disease were monitored monthly by mail and telephone. Lyme borreliosis serologies were done by enzyme-linked immunosorbent assay and Western blot. Rocky Mountain spotted fever serologies were initially screened using Dip-S-Ticks, followed by specific indirect immunofluorescence. Ehrlichiosis serologies were determined by epifluorescent microscopy, as were antibodies to *Babesia microti*.

**RESULTS:** Of the 671 participants, 88 (13%) had antibodies to ≥1 tick-borne organisms, including 34 (5% of the total) with antibodies to *Borrelia burgdorferi*. Twenty-seven participants had evidence of exposure to *B. burgdorferi* at baseline. Seven participants (1%) seroconverted during the course of the study, 5 of whom were symptomatic for Lyme borreliosis. Antibodies to spotted fever group rickettsiae were seen in 28 participants (4%), 22 of whom were positive at baseline and 6 of whom seroconverted during the observation period. None of the seropositive patients had any symptoms or signs of infection. Twenty-four participants (3%) had serologic evidence of exposure to Ehrlichia (all but one to *Ehrlichia equi*); 5 (0.7%) seroconverted during the observation period, including 3 subjects who were asymptomatic. Antibodies to *B. microti* were seen in 7 participants (1%), including one asymptomatic seroconversion during the year of observation. There was evidence of possible dual infection in 5 patients.

**CONCLUSION:** In a high-risk population, there was evidence of exposure to 5 tick-borne pathogens; however, many infections were asymptomatic, and coinfections were rare. **Am J Med. 1999;106:404–409.** ©1999 by Excerpta Medica, Inc.

Of the 10 tick-borne organisms that infect humans in the United States, at least 5 have been reported in residents of New York state. Past studies have suggested that coinfection with >1 organism can occur after a tick bite (1–5). The evaluation of a vaccine preparation to prevent Lyme disease provided a large group of volunteers in which to conduct a serologic study. Baseline seroprevalence and seroconversion, as well as the incidence of multiple infections, were measured for several tick-borne organisms, including the agents of Lyme disease (*Borrelia burgdorferi*), Rocky Mountain spotted fever (*Rickettsia rickettsii*), ehrlichiosis (*Ehrlichia equi* and *Ehrlichia chaffeensis*), and babesiosis (*Babesia microti*).

## METHODS

Participants were recruited in 1994 from a Lyme vaccine study conducted in areas considered endemic for Lyme disease, including Suffolk and Westchester counties of New York. Approximately one-half of the patients had received vaccine (an OspA recombinant protein), and the remainder received placebo. The protocol and consent form were approved by the Institutional Review Board at Long Island Jewish Medical Center.

Participants were required to live or work in a high-risk area for tick-borne infections; have active outdoor exposure, such as gardening, hiking, walking, fishing, picnicking, hunting, or camping in wooded areas; be at least 18 years of age; not be pregnant or planning pregnancy; and be willing to comply with all aspects of the study for a minimum of 1 year. Subjects were excluded if they had symptoms or signs of active arthritis or Lyme disease, were receiving chronic antibiotic treatment or had other chronic illnesses or evidence of impaired immune function.

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After informed consent, subjects had 10 cc of blood collected; serum was aliquoted and stored at −70°C. Patients were contacted monthly by mail and telephone to elicit descriptions of symptoms of tick-borne diseases. If patients reported symptoms of fever, myalgia, fatigue, joint pain, or rashes, they were examined by a study physician and acute and convalescent sera (6 weeks later) were obtained. Additional sera were collected 1 year after the initial visit. If the physician believed that therapy was indicated, treatment with doxycycline 100 mg twice a day for 21 to 30 days was prescribed.

Lyme borreliosis serologies were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) that detected both IgG and IgM (Hillcrest, Cranbury, New Jersey), and an IgG-specific immunoblot test kit (MARDX, Carlsbad, California). For the immunoblot, a 1:1,000 dilution of serum was incubated with a nitrocellulose strip containing the diagnostically important borrelial antigens that had been resolved by electrophoresis before transfer. After a 30-minute incubation, the strip was washed to remove unbound serum and incubated for 15 minutes with alkaline-phosphatase-conjugated antihuman IgG. The strip was washed to remove unbound conjugated antibody and reacted with a precipitating color-developing solution. This resulted in a purple precipitate on antibody-reacted antigen bands. Bands were scored by comparison with the intensity present on the weakly reactive control at 41 kDa. Sera were considered positive if at least five of the 10 (93,66,58,45, 41,39,30,28,23,18 kDa) diagnostic bands were as reactive as the intensity calibrator on the control. All sera were run with negative, weak positive, and positive controls supplied by the manufacturer, as well as internal laboratory positive controls. Testing to confirm seroconversion was done on preserum and postserum samples run simultaneously using a single kit. A positive seroconversion was defined as a positive ELISA and a positive Western blot. If a participant was seropositive at baseline, seroconversion was defined as the presence of two or more new diagnostic bands on the Western blot. All blots were read by someone who was blinded to the clinical information available on the subjects being tested.

Rocky Mountain spotted fever serologies were initially screened for using Dip-S-Ticks (Integrated Diagnostics, Inc., Baltimore, Maryland), a semiquantitative enzyme immunoassay for the detection of rickettsial antibodies. Sera were diluted 1:200 and allowed to react for 5 minutes with a solid membrane that had decreasing concentrations of antigen added at four discrete spots on the support membrane. Strips were washed and the reaction enhanced by the removal of nonspecific sera antibodies. Alkaline-phosphatase-conjugated anti-IgG was allowed to react with bound human antibodies. Next, the strip was transferred to the enzyme substrate reagent that reacted with bound alkaline phosphatase to produce a distinct spot. Each strip contained an internal positive and negative control, as well as a positive control provided by the manufacturer. Strips were scored as nonreactive if no positive dots were seen; weakly reactive if one or two positive dots were seen; and reactive if three or four positive dots were seen. All reactive sera were confirmed by a subsequent immunofluorescent assay as follows: control and patients' sera were diluted to 1:20 in phosphate-buffered saline. This dilution was then used to make twofold serial dilutions. After 20 to 25 microliters of each dilution was applied, slides were incubated at room temperature for 30 minutes. Slides were washed, and each well was overlaid with 25 microliters of diluted conjugate and incubated for 30 more minutes. Slides were then counterstained with Eriochrome Black. After washing, the relative amount of fluorescence was assessed by epifluorescent microscope. The end point was the highest dilution that showed the whole organism. A fourfold increase (to 1:80) was considered a minimal positive result.

Serologic reactivity was determined for both *E. equi* and *E. chaffeensis*. Briefly, *E. equi*–infected equine leukocyte antigen slides were prepared from the blood of experimentally infected horses (provided by John Madigan, University of California, Davis) and *E. chaffeensis* (courtesy of Jacqueline Dawson, Centers for Disease Control, Atlanta, Georgia) was propagated in DH82 cells and used to prepare antigen slides (6). The sera were diluted at the screening dilution of 1:80 in phosphate-buffered saline with 0.5% nonfat dry milk and incubated on individual wells of both *E. equi* and *E. chaffeensis* antigen slides for 1 hour at room temperature in a humidified chamber. Bound antibodies were detected after thorough washing with phosphate-buffered saline by reacting each well with goat antihuman IgG + IgM + IgA conjugate to fluorescein isothiocyanate (Kierkegaard and Perry Laboratories, Gaithersburg, Maryland). After a 1-hour incubation at room temperature, the slides were incubated for 5 minutes in phosphate-buffered saline with 0.005% Evans Blue as counterstain, washed in phosphate-buffered saline, and mounted for epifluorescent microscopy. Each batch of sera tested included known negative and positive control sera obtained from patients convalescent from human monocytic ehrlichiosis (*E. chaffeensis)* or human granulocytic ehrlichiosis (*E. equi*) or from normal subjects with no history of tick-borne disease. The slides were examined for distinct intracellular fluorescence with the morphology and correct distribution of *Ehrlichia* species morulae. A positive sample was any serum that demonstrated specific ehrlichial fluorescence at a dilution of $\geq$1:80. The titer end point was determined as the reciprocal of the highest dilution of sera, to a maximum of 2,560, in which specific ehrlichial fluorescent morphology could still be observed.

Antibodies to *B. microti* were detected by immunoglobulin class-specific indirect immunofluorescence.

**Table 1.** Seroprevalence and Seroconversion (during 1-year follow-up) to *Borrelia burgdorferi, Rickettsia rickettsii, Ehrlichia chaffeensis, Ehrlichia equi,* and *Babesia microti* in 671 Participants

| Pathogen | Total | Previous Exposure, Number (percent) | Seroconverters |
|---|---|---|---|
| *B. burgdorferi* | 34 (5) | 27 (4) | 7 (1) |
| *R. rickettsii* | 28 (4) | 22 (3) | 6 (1) |
| *E. chaffeensis* | 1 (0.1) | 1 (0.1) | 0 |
| *E. equi* | 23 (3) | 18 (3) | 5 (1) |
| *B. microti* | 7 (1) | 6 (1) | 1 (0.1) |
| Multiple | 5 (5) | 5 (5) | 0 |
| Any | 88 (13) | 88 (13) | 0 |

Washed red blood cells from a hamster-grown human-derived strain of *B. microti* was used as antigen. Serum was incubated for 1 hour on a slide containing the *B. microti* at 37°C in a moist chamber. The slides were washed twice in phosphate-buffered saline, incubated with fluorescein-isothiocyanate-conjugated affinity-purified goat antihuman IgG and IgM (Organon-Teknica, Durham, North Carolina) under the same conditions as the primary antibody, washed, and mounted for ultraviolet microscopy. A titer of ≥1:64 in either immunoglobulin class was considered to be reactive.

To assess whether cross-reactivity could have resulted in falsely elevated estimates of the risks of infection, a cross-reactivity study was done. The specimens used were well-defined positives from patients with the following diseases: *R. rickettsii, Rickettsia typhi, Rickettsia prowazekii, Rickettsia conorii, Coxiella burnetii, E. chaffeensis,* and *E. equi*. Titers ≤1:40 were considered negative; those >1:40 were considered evidence of weak cross-reactivity.

## RESULTS

In the spring of 1994, 835 adults 18 years of age and older were enrolled in a 1-year study. All participants lived or worked in high-risk areas of Suffolk (n = 828) or Westchester (n = 7) counties in New York. There were 497 men and 338 women. Their ages were 18 to 72 years (mean 43). One hundred sixty-four patients did not return for the final serum collection; thus, 671 participants remained in the study. Ninety percent of study participants responded to all of the monthly mail and telephone contacts.

Serologic reactivity was assessed using the final serum sample from the 1-year collection. A total of 88 (13%) participants had serologic evidence of infection with ≥1 tick-borne organism. Serologic reactivity was greatest for Lyme borreliosis. Of the 34 subjects (5%) who had antibodies to *B. burgdorferi* (Table 1), 27 had evidence of previous exposure to *B. burgdorferi*. Baseline questionnaires showed that 11 (41%) of the 27 had reported a prior diagnosis of Lyme disease. During the study, 31 participants sought medical attention and were treated for presumed Lyme disease, including 2 participants with evidence of prior infection and 5 with evidence of new infection. Seven subjects (1%) developed antibodies to *B. burgdorferi* during the course of the study, of whom 2 reported episodes of pain in the knees without swelling but associated with myalgia and fatigue. None had a history of rash or tick bites. Three participants who seroconverted had erythema migrans (confirmed by cultures or polymerase chain reaction of biopsy specimens). One of these patients had myalgias, arthralgias, and fatigue, whereas the other 2 denied associated symptoms. The other 2 seroconverters were asymptomatic throughout the period of observation.

Twenty-eight subjects (4%) showed serologic reactivity to Rickettsiae of the spotted fever group in the year-end serum specimen, with six (1%) seroconversions during the observation period. None of the participants had knowledge of past or current Rocky Mountain spotted fever infection, and all were asymptomatic during the observation period. Only 1 recalled a tick bite during the spring of the year that he seroconverted. Subsequent follow-up of another patient revealed an episode of fatigue, myalgia, and arthritic pain that occurred 3 months after his final blood sample was obtained.

There was serologic evidence of exposure to *Ehrlichia* in 24 subjects (4%). All but 1 patient had antibodies to *E. equi*; the remaining patient had evidence of prior exposure to *E. chaffeensis*. Five participants seroconverted to *E. equi* during the observation period. One patient complained of low-grade fevers, fatigue, and myalgia and was treated empirically with oral doxycycline (100 mg twice a day for 21 days) with full resolution of his symptoms. This patient denied knowledge of a prior tick bite. Another patient had a "dog tick" removed in May of 1994; in August of the same year, the patient reported an episode of myalgias and arthralgias associated with fever. The remaining 3 patients reported no symptoms considered typical of ehrlichiosis; however, 1 had an admission for chest pain and another had an attack of gouty arthritis.

Seven participants (1%) had final specimens reactive at the 1:64 dilution for *B. microti*. None of these specimens showed IgM reactivity. The 1 participant who had seroconverted during the study period did not remember a tick bite and was asymptomatic during the year of observation.

### *Dual Infections and Cross-reactivity*

There was evidence of possible dual infection in 5 participants (Table 2), 3 of whom had evidence of prior exposure to both pathogens in their baseline sera.

The results of the cross-reactivity tests are listed in

**Table 2.** Antibodies to Lyme, *Borrelia burgdorferi, Rickettsia rickettsii, Ehrlichia chaffeensis, Ehrlichia equi,* and *Babesia microti* in Patients with Evidence of More Than One Exposure

| Patient Number | *B. burgdorferi* | *R. rickettsii* | *E. equi* | *E. chaffeensis* | *B. microti* |
|---|---|---|---|---|---|
| 1 | | Baseline positive | Seroconverted | | |
| 2 | | Baseline positive | | Baseline positive | |
| 3 | Baseline positive | | Baseline positive | | |
| 4 | Baseline positive | Baseline positive | | | |
| 5 | Baseline positive | Seroconverted | | | |

Table 3. There was cross-reactivity at titers of ≤1:40 between *R. prowazekii* and *E. chaffeensis*. There was no cross-reactivity at titers of ≤1:40 seen between sera known to be positive for Ehrlichia with *Rickettsia*.

## DISCUSSION

Previous studies have examined the prevalence of tick-borne diseases in at-risk populations (1–18). Lyme disease is the most commonly reported tick-borne disease in the United States, with most of the cases reported from Connecticut, Rhode Island, New York (especially Suffolk and Westchester counties), New Jersey, Delaware, Pennsylvania, Wisconsin, and Maryland. The tick most commonly implicated in transmission of the agent of Lyme disease (*B. burgdorferi*) is from the genus Ixodes. Other infectious agents that may be transmitted by this tick include *B. microti* and the agent of human granulocytic ehrlichiosis (*E. equi*; 3,19,20). In the United States *R. rickettsii*, the agent of Rocky Mountain spotted fever, is usually transmitted by a different vector, the American dog tick (*Dermacentor variabilis* and *andersoni*). The cause of human monocytic ehrlichiosis (*E. chaffeensis*) is transmitted by the Lone Star tick *(Amblyomma americanum)* (8,11).

**Table 3.** Cross-reactivity of Serum Samples Known to Be Positive for Selected Diseases

| | Cross-reactivity to | | |
|---|---|---|---|
| Known Disease (titer) | *E. chaffeensis* | *E. equi* | *R. rickettsii* |
| *R. rickettsii* ≤1:128 | <1:40 | <1:40 | |
| *R typhi* ≤1:512 | <1:40 | <1:40 | |
| *R. prowazekii* 1:2,560 | ≤1:40 | <1:40 | |
| *R. conorii* 1:512 | <1:40 | <1:40 | |
| *R. conorii* ≤1:1,024 | <1:40 | <1:40 | |
| *C. burnetii* 1:1,024 | <1:40 | <1:40 | |
| *C. burnetii* 1:256 | <1:40 | <1:40 | |
| *E. chaffeensis* 1:512 | | <1:40 | <1:40 |
| *E. chaffeensis* >1:1,280 (IgG) | | <1:40 | |
| *E. equi* 1:160 | <1:40 | | |
| *E. equi* ≤1:512 | <1:40 | | <1:40 |
| *E. equi* 1:320 (IgG) | <1:40 | | |

Theoretically, one bite from a multiply infected Ixodes tick could transmit up to 3 infectious agents. A person could also be coinfected by different tick bites. Many of our participants reported multiple tick bites per week during the spring and summer months. Ideally, each tick would have been collected, identified, and examined for the presence of organisms. This was not feasible in this study.

In the United States in 1995, the reported incidence of Lyme disease was 4.4 cases per 100,000 population; the risk in New York state was about five times greater (18,21). As our participants were chosen because they were thought to be at high risk of contracting disease, the proportion of seropositives (5%) was not surprising. The rate of seroconversion during a 1-year period (1%) may be misleadingly low, as one-half of the participants received a vaccine the efficacy of which may be >80% in patients under 60 years of age (22). Even if the vaccine were completely protective, the rate of seroconversion would have been about 2%. It is also possible that presumptive treatment for Lyme disease may have rendered some infected patients seronegative. Two of the 7 seroconverters were asymptomatic, whereas almost 60% of the participants with serologic evidence of past Lyme disease had no recollection of the disease's symptoms. However, participants had been instructed to report any evidence of disease that occurred during the study and may have been sensitized to symptoms that might otherwise have been ignored.

From 1981 to 1992, the incidence of Rocky Mountain spotted fever was 0.6 to 1.5 cases per 100,000 in the United States (8). Approximately 5% to 12% of high-risk populations have evidence of antibodies, with an annual seroconversion rate of 2% to 5% (1). We had fewer patients with preexisting antibodies (4%) and seroconversions (1%). However, the reported incidence of Rocky Mountain spotted fever has decreased steadily during the last decade and can fluctuate from year to year. It is not surprising that all 6 seroconverters in our study were asymptomatic (1,11,12,14).

Veterinary ehrlichioses have been described for >60 years, but recognition of ehrlichial infections in humans is relatively recent. *E. chaffeensis,* first described in 1990,

infects mostly mononuclear phagocytes. This ehrlichia is genetically and antigenically related to *E. canis* and causes disease mostly in the southeastern and south-central United States. Since then, an ehrlichia closely related to *E. equi* and *Ehrlichia phagocytophila* that infects mostly granulocytes has been identified as the causative agent of human granulocytic ehrlichiosis. This infection occurs predominantly in upper midwestern and northeastern states, including Westchester and Suffolk counties in New York, where this study was conducted (20,24–30).

The incidence of ehrlichioses in the United States is low (1 per 100,000 per year) but may be as high as 14 to 16 cases per 100,000 in endemic areas. These results span the years 1985 to 1990 and probably represent serious cases of the disease (22,23). As ehrlichioses are not reportable in most states, the true incidence may be higher. In military personnel, seroconversion rates from 0.5% to 11% have been reported after field maneuvers in a high-risk area; many of these seroconversions were asymptomatic (1). All of our participants' seroconversions were due to *E. equi* and most were asymptomatic.

The incidence of babesia exposure in our study was lower than previously reported. We found 1% of participants to be seropositive, and only 1 person seroconverted during the year-long surveillance period. Previous investigators have reported that as many as 5% of subjects have antibodies to this tick-borne protozoal parasite (2,3,17,19).

A reassuring finding was the relatively low frequency of multiple infections with tick-borne pathogens. In contrast to previous reports in residents of Rhode Island, southern New England, and New York, which described frequent coinfections in patients with Lyme disease, we found no evidence of multiple infections during the 1-year surveillance period (2–6). Even though some of our participants had serologic evidence of past infection with $\geq$1 pathogen, we could not determine when these infections occurred or if they were concurrent. In those with evidence of multiple infections who had seroconverted during the observation period, all had previous infections, suggesting sequential rather than simultaneous infection. However, most of our participants were treated with doxycycline if there was any suspicion of infection. Thus, the seroconversion rates for *E. chaffeensis*, *E. equi,* and *R. rickettsii* may have been greater had patients been followed and been treated less aggressively. Because doxycycline is not an effective therapy for babesiosis, the seroconversion rate for babesia is probably a valid estimate. As a percentage of the total number of participants with any evidence of a tick-borne infection, only 6% (5 of 88) had evidence of multiple infections. The rate of multiple infections is likely to be even lower in areas where individual infections are infrequent.

Serologic studies cannot determine whether the detected antibodies were stimulated by the specific infectious agent being assayed, or whether the activity is the result of infection by a related organism. There have been reports of patients with ehrlichiosis who developed serologic reactions for *B. burgdorferi* without clear clinical evidence of Lyme disease (31). The coexistence of Ehrlichia antibodies and *B. burgdorferi* antibodies may represent two infections (32). However, evidence of any tick-borne infection is an excellent marker of tick exposure and risk of other tick-borne diseases. It is also possible that some of these serologic responses result from a tick bite that leads to inoculation with antigenically similar nonpathogenic organisms. Also, serologic reactions to $>$1 tick-borne agent could result from true serologic cross-reactions or nonspecific induction of antibody responses for undetermined reasons. Although Ehrlichia infections can induce a polyclonal gammopathy, and autoimmune antibodies may occasionally be present during human granulocytic ehrlichiosis, it is not clear how *E. equi* could induce antibodies to *B. burgdorferi*. Indeed, we found that although some participants had antibodies to *E. equi*, *B. burgdorferi,* or *Babesia microti,* very few had dual infections**.** This is a strong indicator that serologic cross-reactivity does not occur and that these reactions reflect a specific antigen-driven immunologic reaction. Some of the *R. rickettsii* and *Ehrlichia* species' seroreactivity might be because of other "nonpathogenic" infections with similar organisms. Testing showed minimal cross reactivity between *R. prowazekii* and *E. chaffeensis,* and between *E. chaffeensis* and *E. equi*. These results would not be considered clinically significant, nor would they have been included as positives in this seroprevalence study.

In summary, a 1-year seroconversion study of patients residing in New York state who were at high risk for tick-borne diseases showed that approximately 13% had evidence of infection with *B. burgdorferi, R. rickettsii, E. equi, E. chaffeensis,* or *B. microti*. Most of the patients who seroconverted had no symptoms. The incidence of coinfection was low. Clinicians should maintain a cautious approach to patients with symptoms or signs of a tick-borne disease, especially if the infection is unusually severe. Fortunately, doxycycline has excellent antimicrobial activity against 4 of the 5 pathogens discussed and should be used whenever possible.

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