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# Infection with Agents of Human Granulocytic Ehrlichiosis, Lyme Disease, and Babesiosis in Wild White-Footed Mice (*Peromyscus leucopus*) in Connecticut

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**White-footed mice, *Peromyscus leucopus*, were captured in southern Connecticut during 1997 and 1998 to determine the prevalence of infections caused by granulocytic *Ehrlichia* sp., *Borrelia burgdorferi*, and *Babesia microti*. Of the 50 mice captured and recaptured, 25 of 47 (53.2%) and 23 of 48 (47.9%) contained antibodies to the BDS or NCH-1 *Ehrlichia* strains, respectively, as determined by indirect fluorescent antibody (IFA) staining methods. The majority (83.3%) of 48 mice also contained antibodies to *B. burgdorferi*, as determined by enzyme-linked immunosorbent assay. Moreover, 20 of 26 (76.9%) contained antibodies to *B. microti* by IFA staining methods. In nested PCR tests using the 16S rRNA gene, the DNA of the human granulocytic ehrlichiosis (HGE) agent was detected in 17 of 47 mice (36.2%), but only 4 (23.5%) of these 17 mice were PCR positive at each capture. Antibody-positive reactions to granulocytic *Ehrlichia* sp. organisms were detected in 17 of 23 (73.9%) of the PCR-positive mice. The sequences from PCR products from nine positive blood samples were identical to the HGE agent. *Ehrlichia* spp. were cultured from three of five mice captured in April 1998, including one that was PCR positive in April 1997. In addition, 2 of 14 larval *Ixodes scapularis* pools, which were attached to two PCR-positive mice, contained DNA of the HGE agent. A high percentage of white-footed mice are infected or have been infected naturally by the HGE agent with low-level persistent infection or frequent reinfection in some individual mice. However, the changes noted in the presence of DNA and antibodies in repeated blood and serum samples from individual mice over several months of field collection suggests that infection with granulocytic *Ehrlichia* is transient in most wild *P. leucopus*.**

Human granulocytic ehrlichiosis (HGE) is a newly recognized, tick-associated febrile illness of the northeastern and upper midwestern United States that was first described in 1994 from cases in Minnesota and Wisconsin (5). The pathogen for HGE is very closely related to *Ehrlichia phagocytophila* and *Ehrlichia equi* (30). The distribution of this disease in the northeastern and midwestern United States overlaps with that of the black-legged tick, *Ixodes scapularis*, the vector for *Borrelia burgdorferi* and *Babesia microti*, the agents of Lyme borreliosis and human babesiosis (4, 30), respectively. Antibodies to multiple tick-borne agents, including granulocytic ehrlichiae and *Ehrlichia chaffeensis*, the agent of human monocytic ehrlichiosis, have been detected in human patients with Lyme borreliosis (17, 20).

Evidence for *I. scapularis* as the vector of the HGE agent is substantial. The DNA of the HGE agent has been detected in *I. scapularis* from Connecticut, Wisconsin, New York, Massachusetts, and Rhode Island (21, 24, 26, 29, 33). Field-collected *I. scapularis* nymphs transmitted the HGE agent to uninfected mice in the laboratory, and nymphs fed on these mice as larvae also transferred infection (9).

Similarly, the white-footed mouse, *Peromyscus leucopus*, appears to be a reservoir for granulocytic ehrlichiae. The DNA of

the HGE agent or antibodies to this bacterium have been detected in this rodent in Connecticut, Minnesota, and Rhode Island (15, 32, 33). Larval *I. scapularis* that had fed on *P. leucopus* were shown to acquire the HGE agent and transmit the pathogen after molting to nymphs (29). However, little is known about the dynamics of the HGE agent in wild mice and its relationship to other tick-associated pathogens. Antibodies to multiple tick-borne agents of ehrlichiosis as well as babesiosis and Lyme borreliosis have been detected in the white-footed mouse (15, 20). The present study reports the prevalence of white-footed mice with antibodies to the HGE agent, *B. burgdorferi*, and to *Babesia microti* through a season of activity for nymphal and larval *I. scapularis*. The detection of the DNA of granulocytic ehrlichiae in this rodent over time and in feeding *I. scapularis* and the culture of the HGE agent from *P. leucopus* also are reported.

## MATERIALS AND METHODS

**Samples.** *P. leucopus* mice were trapped live at various intervals (roughly monthly) from 16 April through 23 September 1997, and in April 1998, using Sherman box traps (H. B. Sherman Traps, Inc., Tallahassee, Fla.) at private residences in the three towns of East Haddam, Lyme, and Old Lyme, Conn. Mice also were trapped at a forested, urban focus for Lyme borreliosis in Bridgeport, Conn. (16). Mice were anesthetized with methoxyflurane, ear tagged, and examined for ticks. Attached *I. scapularis* organisms were removed with forceps, placed in vials, and returned to the laboratory. Duplicate samples of clotted blood and EDTA-whole blood were obtained, in most cases, from each white-footed mouse in accordance with protocols approved by the Connecticut Agricultural Experiment Station's institutional animal care and use committee. Serum was obtained after centrifugation of clotted blood samples at 2,000 × *g* for

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TABLE 1. Numbers of mice captured and ticks collected at four locations in Connecticut in 1997

| Capture site | No. of mice captured or recaptured | No. of captured individuals | Trapping period | No. of *I. scapularis* collected | |
|---|---|---|---|---|---|
| | | | | Larvae | Nymphs |
| Old Lyme[a] | 19 | 13 | 30 April–27 August[a] | 223 | 22 |
| Lyme | 12 | 9 | 23 April–17 July | 39 | 4 |
| East Haddam | 12 | 5 | 16 April–16 September | 38 | 20 |
| Bridgeport | 45 | 23 | 20 June–19 September | 673 | 33 |
| Total | 88 | 50 | | 973 | 79 |

[a] Five more mice were trapped in April 1998 at Old Lyme, Conn.

30 min and stored at $-60°C$ until antibody analyses could be conducted. EDTA-blood was stored at $4°C$ for analysis by PCR. In East Haddam, Lyme, and Old Lyme, ear tissue samples were taken from anesthetized mice by punch biopsy (27), placed into sterile phosphate-buffered saline (PBS) solution, and returned to the laboratory for culturing of *B. burgdorferi*.

**Serological assays.** Indirect fluorescent antibody (IFA) staining methods were used to detect total immunoglobulins to the BDS or NCH-1 strains of granulocytic ehrlichiae, Arkansas strain of *E. chaffeensis* (DH 82 cells), and *Babesia microti*. The BDS strain is a human isolate of the HGE agent from Wisconsin maintained in horse-infected neutrophils. The NCH-1 strain, human isolate of this organism from Nantucket, Massachusetts, is grown in human promyelocytic leukemia cell (HL-60; American Type Culture Collection CCL-240) cultures at Yale University. In addition, 31 mice were tested at least once with the Webster strain of the HGE agent ($n = 46$ serum samples), a Wisconsin human isolate cultivated in HL-60 cells (3). Details of the source and use of fluorescein-conjugated antibodies, negative and positive control sera, the specificity of antibody tests, and the method of conservative grading of fluorescence of morulae have been reported previously (15, 21). Briefly, infected cells were fixed to glass microscope slides by acetone treatment. These cells were used along with fluorescein isothiocyanate-labeled goat anti-*P. leucopus* immunoglobulin (H- and L-chain specific) prepared by Kirkegaard & Perry Laboratories, Inc. (Gaithersburg, Md.) and diluted to 1:20 in PBS solution. Sera from seven white-footed mice born in the laboratory and unexposed to ticks or tick-borne agents were used as negative controls, while serum from an inoculated C3H/HeN mouse (28) was used as an additional positive control. Uninfected lysates of HL-60 cells were included as controls in parallel tests of all sera tested with preparations of infected HL-60 cells with a distinct reactivity of ehrlichial morulae, with a serum dilution of $\geq 1:80$ considered positive. Antigen of *Babesia microti* consisted of infected erythrocytes obtained from Golden Syrian hamsters that had been inoculated with whole blood from an infected person diagnosed with human babesiosis. Details on the source of fluorescein-conjugated antibodies and procedures for preparing the antigen and have been described (2, 15). Distinct reactivity of infected erythrocytes at a serum dilution of $\geq 1:80$ was considered positive for antibodies.

An enzyme-linked immunosorbent assay (ELISA) was used to detect antibodies to *B. burgdorferi* in mouse serum samples. This assay was developed earlier to quantitate total concentrations of antibody to *B. burgdorferi* 2591 (14). An affinity-purified peroxidase-labeled goat anti-*P. leucopus* immunoglobulin (H- and L-chain specific), was purchased from Kirkegaard & Perry Laboratories and diluted to 1:2,000 in a mixture of PBS solution, donor horse serum (Gibco Laboratories, Gaithersburg, Md.), and dextran sulfate sodium salt (Sigma Chemical Co., St. Louis, Mo.).

**Culture for *B. burgdorferi*.** Ear tissue was collected from anesthetized mice by punch biopsy, placed into microcentrifuge tubes containing sterile PBS solution, and returned to the laboratory. Tissue samples were introduced into duplicate tubes of Barbour-Stonner-Kelly medium with or without 0.1% agarose gel as previously described (1, 2). Cultured spirochetes were identified by using IFA staining methods with murine monoclonal antibody H5332 directed to outer surface protein A of *B. burgdorferi*.

**PCR analysis for ehrlichiae.** A nested PCR assay described previously (22) was used to detect the DNA of granulocytic ehrlichiae in EDTA whole blood from the white-footed mice and larval and nymphal *I. scapularis* removed from these animals. Total DNA was purified from 200 μl of EDTA whole blood with the QIAamp blood purification kit (Qiagen Inc., Chatsworth, Calif.) following the manufacturer's suggested protocol. Purified DNA was eluted from the columns in 200 μl of distilled $H_2O$ ($dH_2O$) and stored at $4°C$ until used as a template for PCR amplification. DNA was extracted from *I. scapularis* larvae by using the QIAamp tissue kit protocol. Larval pools (representing all the ticks from a single mouse captured on a particular date) or individual nymphs were crushed in 1.5-ml microcentrifuge tubes containing 180 μl of a tissue lysis buffer (Qiagen Inc.). Following proteinase K digestion and column purification, the DNA was eluted in 100 μl of $dH_2O$.

PCR amplifications of the *Ehrlichia* 16S rRNA gene (rDNA) were performed in a Perkin-Elmer 9600 thermal cycler (Perkin-Elmer Applied Biosystems, Fos-

ter City, Calif.) using a nested protocol as previously reported (22). The primary reactions used primer set ge3a and ge10r, and ge9 and ge2 were used for the nested reactions. Positive and negative controls were extracted and amplified by PCR methods in parallel with all specimens. Reaction products were maintained at $4°C$ until analyzed by agarose gel electrophoresis or purified for DNA sequencing.

DNA sequencing reactions included fluorescence-labeled dideoxynucleotide technology (d-rhodamine Cycle Sequencing Ready Reaction Kit; Perkin-Elmer). Sequencing reaction products were separated, and data were collected with an ABI 377 automated DNA sequencer (Perkin-Elmer).

**Culture and second PCR analysis for ehrlichiae.** EDTA–whole-blood samples (50 to 100 μl) from five white-footed mice captured in Old Lyme in April 1998 were added to duplicate sets of flasks containing HL-60 cells and cultured as previously described (12). One set of culture flasks contained ampicillin to prevent contamination problems. Light microscopy slides of cultured HL-60 cells were air-dried, stained with Diff-Quik (Baxter Healthcare Corp., Miami, Fla.), and examined for infection. The 8F and 9B primers based on the sequence of the p-44 gene that encodes the immunodominant 44-kDa protein of the HGE agent (11) were used in the PCR assay of cultured ehrlichiae as described previously (19). The denaturing, annealing, and extension temperatures were 94, 55, and $72°C$, respectively, for 1 min at each step for 30 cycles.

**Statistical analysis.** The *z* test was used to compare the proportion of positive mice between two groups of interest within the same category with the Yates correction factor applied to the calculations (SigmaStat; SPSS Inc., Chicago, Ill.). The proportion of mice with antibodies to *B. burgdorferi* was compared with that of those with immunoglobulins to *Babesia microti*, BDS antigen, or NCH-1 antigen. The proportion of mice with antibodies to these antigens early in the tick season was compared with that obtained late in the year (August and September) in the Lyme area and Bridgeport.

## RESULTS

Fifty individual white-footed mice were captured or recaptured 88 times at the four Connecticut locations during 1997. In addition, five mice were captured in Old Lyme in April 1998, including one mouse first captured in April 1997. Twelve mice were captured twice, five mice were captured three times, two mice were trapped five times, and one mouse in East Haddam was captured six times. Eighty-two serum samples and 81 EDTA-blood samples were collected from 48 of 50 individual white-footed mice (Table 1). *I. scapularis* larvae ($n = 973$) and *I. scapularis* nymphs ($n = 79$) were removed from 80 mice. Unadjusted for seasonal trends, mean infestations of larval and nymphal *I. scapularis* ($\pm$ standard errors of the means) on the mice at capture were $12.0 \pm 1.8$ (range, 0 to 99) and $0.9 \pm 0.2$ (range, 0 to 12), respectively. One-quarter of the mice ($n = 22$) had cofeeding larval and nymphal *I. scapularis* ticks at the time of capture.

Antibodies to granulocytic ehrlichiae, *B. burgdorferi*, or *Babesia microti* were detected in 42 of the 48 white-footed mice at some point during the trapping period. At least one serum sample from over half of the mice contained antibodies to the BDS strain (53.2% of 47 mice) and NCH-1 strain (47.9% of 48 mice) (Table 2). Results were concordant for 44 (93.6%) of the 47 mice. Three mice were positive only to BDS antigen in the August samples. There was no difference in the proportion of mice with ehrlichial antibodies to either or both strains of

TABLE 2. Number of white-footed mice tested, reactivities of their sera to *B. burgdorferi*, the HGE agent (BDS and NCH-1 strains), and *B. microti* by ELISA or IFA staining methods, and number of mice positive for the HGE agent by PCR

| Capture site | No. of mice tested | No. (%) of mice with antibodies to: | | | | No. (%) of mice testing positive for HGE agent by PCR |
| --- | --- | --- | --- | --- | --- | --- |
| | | *B. burgdorferi* | *B. microti* | BDS | NCH-1 | |
| Total Lyme area | 26 | 20 (76.9) | 20 (76.9) | 13 (50.0) | 11 (42.3) | 11 (42.3) |
| Old Lyme | 12 | 10 (83.3) | 11 (91.7) | 9 (75.0) | 7 (58.3) | 8 (66.7) |
| Lyme | 9 | 6 (66.7) | 7 (77.8) | 2 (22.2) | 2 (22.2) | 1 (11.1) |
| East Haddam | 5 | 4 (80.0) | 2 (40.0) | 2 (40.0) | 2 (40.0) | 2 (40.0) |
| Bridgeport | 22[a] | 20 (90.9) | 3 (14.3) | 12 (57.1) | 12 (54.5) | 6 (27.3) |
| Total | 48[a] | 40 (83.3) | 23 (48.9) | 25 (53.2) | 23 (47.9) | 17 (36.2) |

[a] Twenty-one mice were tested for antibodies with BDS and *B. microti* antigen and for the HGE agent by PCR. A total of 47 were tested with BDS and *B. microti* antigen and by PCR.

antigen between the three-town Lyme area and Bridgeport ($z = 0.0721$, $P = 0.943$). Antibodies to *E. chaffeensis* were detected in two serum samples from 2 of 47 mice at titers of 1:80 and 1:320. Sera from these two mice also were reactive to the BDS (1:80 and 1:630) and NCH-1 antigen (1:640 and 1:320).

The proportion of mice with *B. burgdorferi* antibodies (80.3% of 48 mice) was significantly greater than the percentage of mice testing positive to either ehrlichial strain ($z = 2.288$, $P = 0.022$). Titers of antibody to *B. burgdorferi* ranged from 1:320 to 1:40,960. All of the mice that had immunoglobulins to either or both granulocytic ehrlichial strains also contained antibodies to *B. burgdorferi*. In southeastern Connecticut, seropositivity rates for *Babesia microti* were also high (76.9% of 26 mice) (Table 2). There was no difference in the proportion of mice with immunoglobulins to *B. burgdorferi* and *Babesia microti* in the Lyme area ($z = -0.375$, $P = 0.708$). However, only 3 of 21 mice (14.3%) contained antibodies to *Babesia microti* in Bridgeport. The proportion of mice with serum antibodies to *B. burgdorferi* increased steadily through the summer from 42.9% (of 7 mice) in April to 75% (of 8 mice) in June to 100% (of 8 mice) by September. A similar trend was observed for *Babesia microti* in the three-town Lyme area. All of the mice captured in September had detectable antibodies against *B. burgdorferi* and, in the Lyme area, against *Babesia microti*. Nevertheless, the proportion of seropositive mice in the early season (April and May) was not significantly different from that at the end of the season (August and September) for either *B. burgdorferi* ($z = 1.383$, $P = 0.164$) or *Babesia microti* ($z = 1.383$, $P = 0.164$). In the three-town Lyme region, antibodies for at least one ehrlichial strain, *B. burgdorferi*, and *Babesia microti* were found in 12 (46.2%) of the mice.

Using the nested primer sets, there were 23 blood samples that contained the DNA of the HGE agent, representing 17 individual mice that were captured in 1997. Seventeen (73.9%) of the PCR-positive samples also had antibodies to either or both of the two granulocytic ehrlichial strains. The nucleotide sequence for the products amplified from nine of the positive mice (an approximately 400-bp region of the 16S rDNA) was identical to the corresponding published sequence for the HGE agent (8). Six mice were PCR positive yet lacked detectable antibodies to the BDS or NCH-1 antigen. However, two of those mice, which were captured only once, were serologically reactive to the Webster strain (1:160).

Cultures of HGE bacteria from the EDTA blood samples of three of the five mice captured in Old Lyme in April 1998 were positive. While the flasks of HL-60 cells without ampicillin were contaminated, three of the other culture sets contained the HGE agent. The identity of this organism was confirmed in

these three cultures by PCR using primers for the p-44 gene sequence. Serum was available for two of these mice, i.e., one HGE culture-positive (no. 352) and one culture-negative mouse. No reactivity to the NCH-1 strain was noted for these two mice, but immunoglobulins to *Babesia microti* (1:1,280) were detected in mouse 352. One of the culture-positive mice (no. 317) was captured previously on three occasions in 1997. Serum and whole blood samples from this animal lacked ehrlichial antibodies to and the DNA of the HGE agent in April. The mouse was seropositive and PCR positive in June and September.

The Lyme disease spirochete was successfully cultured from 12 of the 27 mice captured in the three-town Lyme area. Six individual mice on nine separate occasions from April through September were culture positive for *B. burgdorferi* and PCR positive for the HGE agent. Changes in the proportion of mice with antibodies to ehrlichiae or with ehrlichial DNA through the season were less than those observed for *B. burgdorferi* or *Babesia microti*. The proportion of IFA- or PCR-positive mice never exceeded 80% (of five mice in August). Initially, a low proportion of the mice showed evidence of previous infection by the HGE agent by having antibodies (14.3% of seven mice in April and 16.7% of six mice in May) or DNA (28.6% in April and 0% in May) present. Only three mice (37.5%) were PCR positive in September in contrast to six mice (75%) with antibodies. Again, the proportion of antibody- or PCR-positive mice in the early tick season and late season was statistically insignificant ($z = 0.863$, $P = 0.388$ and $z = 0.157$, $P = 0.875$, respectively).

The proportion of mice with past and current infections with any or all of the agents of HGE, Lyme disease, and human babesiosis was high, based on all methods utilized in this study (IFA, ELISA, PCR, and culture) (Table 3). Over 80% of the mice in the Lyme area and over 90% of the animals in Bridgeport showed infection by at least one pathogen. Both *B. burgdorferi* and *Babesia microti* had infected nearly three-quarters of the mice in the Lyme area, and half of these animals showed evidence of infection by all three pathogens. For both 1997 and 1998, a total of 33 of 53 mice (62.2%) showed evidence of past or current infection with granulocytic *Ehrlichia*.

There were 17 mice for which multiple serum and EDTA blood samples were obtained, nine from Bridgeport and eight from the three-town Lyme area. Most of these mice ($n = 11$) were recaptured only once. The majority (10 of 17) of the mice were seropositive for a granulocytic *Ehrlichia* upon initial capture and four others had seroconverted from negative to positive upon subsequent capture. There were no serologic reversions (changes from positive to negative). Three mice caught nine times in Bridgeport never had detectable ehrlichial anti-

TABLE 3. Number and proportion of white-footed mice captured in 1997 with evidence of past or current infections with *B. burgdorferi*, the HGE agent, and *B. microti* by either ELISA, IFA staining methods, PCR, or culture[a]

| Capture site | No. (%) of mice with evidence of infection with: | | | | |
|---|---|---|---|---|---|
| | Any of the three pathogens | *B. burgdorferi* and HGE agent | *B. microti* and HGE agent | *B. burgdorferi* and *B. microti* | All three pathogens |
| Lyme area | 23 (85.2) | 14 (53.8) | 15 (57.7) | 19 (73.1) | 13 (50.0) |
| Bridgeport | 20 (90.9) | 15 (68.2) | 3 (14.3) | 3 (14.3) | 2 (9.5) |
| Total | 43 (87.8) | 29 (60.4) | 18 (31.6) | 22 (46.8) | 15 (31.9) |

[a] Not included in the table are the five mice captured in April 1998, of which three were PCR positive for the HGE agent. Serum was available for two of these mice. Antibodies to *B. burgdorferi* were detected in two mice, antibodies to *B. microti* were detected in one mouse, and antibodies to the NCH-1 strain of the HGE agent were detected in one mouse.

body titers of ≥1:80 to the BDS or NCH-1 antigen. By contrast, only four (23.5%) of the mice were PCR positive at each capture and six (35.3%) changed from positive to negative ($n = 2$), from negative to positive ($n = 3$), or both ($n = 1$). The sixth mouse was PCR negative, PCR positive, and then negative for the remainder of the season (end of July through September). The DNA of the HGE agent was not detected in the blood of seven (41.2%) of the mice, although five of these rodents had antibodies to a granulocytic *Ehrlichia*.

Two mice (no. 317 and 325) could be followed through most of the tick season (April to September) (Table 4). Titration endpoints for *B. burgdorferi* antibodies for one male white-footed mouse (no. 325), captured in East Haddam on six occasions, increased steadily through the summer. Titers went from 0 in April to 1:40,960 in September. Concentrations of antibodies to *Babesia microti* also increased, while titers of immunoglobulin for ehrlichial antibodies increased and then declined by September. This mouse was initially PCR positive and serologically negative in the early spring and, subsequently, was PCR negative and serologically positive. The mice numbered 305 and 317 were negative serologically and by PCR upon initial capture and became positive later (Table 4).

Of 14 larval pools of *I. scapularis* tested for ehrlichial DNA by PCR methods, 3 were positive. Two of the mice (no. 305 and 317), from which the positive larvae were obtained, initially lacked antibodies and ehrlichial DNA but contained antibodies and bacterial DNA when the larval ticks were removed (27 June for mouse 317 with 12 larvae and 27 August for mouse 305 with 58 larvae). The third mouse with bacterial-DNA-positive *I. scapularis* larvae attached was captured in Bridgeport and had 23 larvae and eight nymphs attached on 20 June. This mouse lacked bacterial DNA during both captures on 20 June and 1 July but had antibodies on both occasions to the BDS, NCH-1, and Webster strains at titers of 1:160 to 1:1,280 for June and July. However, one of the eight nymphs removed from this mouse contained the DNA of the HGE agent.

## DISCUSSION

The majority of the *P. leucopus* mice in this study showed evidence of past or current infection with multiple tick-borne agents. A high proportion of white-footed mice in Connecticut were exposed to and infected by granulocytic ehrlichiae, Lyme disease spirochetes, and, in some areas, *Babesia* organisms. Antibodies to granulocytic ehrlichiae, DNA of the HGE agent in the peripheral blood, or cultivable *Ehrlichia* in Connecticut were detected in many of the mice (62.2% of 53 mice). This was especially true in Old Lyme, where 74% of *P. leucopus* mice contained antibodies to ehrlichiae and 36.2% of the mice had DNA of the HGE agent at some point through the spring,

TABLE 4. Serological and PCR results for three white-footed mice for granulocytic ehrlichiae (BDS or NCH-1 strains), *B. microti*, and *B. burgdorferi* by ELISA or IFA staining from April to September 1997

| Mouse | Test | Antibody titer or PCR result for[a]: | | | | | |
|---|---|---|---|---|---|---|---|
| | | April | May | June | July | August | September |
| 305 | Ehrlichia PCR | | | Neg. | | Pos.[c] | |
| | Ehrlichia BDS | | | Neg. | | 1:80 | |
| | Ehrlichia NCH-1 | | | Neg. | | Neg. | |
| | *B. burgdorferi* | | | Neg. | | Neg. | |
| | *B. microti* | | | Neg. | | 1:640 | |
| 317[b] | Ehrlichia PCR | Neg. | | Pos.[c] | | | Pos. |
| | Ehrlichia BDS | Neg. | | 1:1,280 | | | 1:160 |
| | Ehrlichia NCH-1 | Neg. | | 1:640 | | | 1:80 |
| | *B. burgdorferi* | Neg. | | 1:2,560 | | | 1:10,240 |
| | *B. microti* | Neg. | | 1:80 | | | 1:160 |
| 325 | Ehrlichia PCR | Pos. | Neg. | Neg. | Neg. | | Neg. |
| | Ehrlichia BDS | Neg. | 1:320 | 1:640 | 1:320 | | 1:80 |
| | Ehrlichia NCH-1 | Neg. | 1:160 | 1:160 | 1:2,560 | | 1:1,280 |
| | *B. burgdorferi* | Neg. | 1:1,280 | 1:1,280 | 1:5,120 | | 1:40,960 |
| | *B. microti* | Neg. | 1:160 | 1:160 | 1:160 | | 1:640 |

[a] Pos., positive; Neg., negative.
[b] The HGE agent was cultured in HL-60 cells from the EDTA-blood of this mouse after recapture on 22 April 1998 in Old Lyme, Conn.
[c] Larval *I. scapularis* removed from the mouse were positive for granulocytic ehrlichiae by PCR.

summer, or fall. Immunoblots of 19 of 20 samples from these mice also were positive and showed distinct reactivity with the 44-kDa protein (20). By contrast, only 15% of the *P. leucopus* mice from near the home of the Rhode Island index HGE case were IFA positive for HGE antibodies (33), which may reflect a lower intensity of transmission. The detection and isolation of the HGE agent in *P. leucopus* and the detection of the HGE agent in larval *I. scapularis* removed from PCR-positive mice in this study indicate that this organism may be the primary ehrlichial agent in Connecticut. Few mice in this study had antibodies to *E. chaffeensis*. Antibodies reactive to *E. chaffeensis* have been detected previously in serum samples from white-footed mice and humans in Connecticut (15, 18), but culture- or PCR-confirmed cases of human monocytic ehrlichiosis are lacking. While these reactions could represent cross-reactivity between the agent of HGE and *E. chaffeensis*, there appears to be no cross-reactivity with *E. chaffeensis* at the titers of HGE antibody detected in these mice (10, 23).

The pattern of antibody response and patency of infection of ehrlichiae in serially captured mice appear to show initial infection in some animals. Initial infection during the summer tick season is suggested by antibody test results and PCR findings in the mid- to late summer for two mice that were previously PCR and serologically negative. Mice that contained ehrlichial DNA but lacked antibodies to ehrlichiae at capture may have had recent infection. Immune response to infection in laboratory mice is rapid (12). The strongest immunoglobulin M response and detection of the immunoglobulin G response to the 44-kDa antigen in C3H mice infected with the HGE organism by tick bite was at day 10 after tick feeding. Morulae were detectable in mouse neutrophils within 5 days of tick repletion and confirmed by PCR methods. In this study, *P. leucopus*, upon seroconversion, had antibodies to ehrlichial antigens throughout the summer.

Most of the mice in this study also carried antibodies to *B. burgdorferi* and all the mice with antibodies to the HGE agent contained antibodies to *B. burgdorferi*. The majority of the mice from all sites in this study except Bridgeport also carried antibodies to *Babesia microti*. Given the low prevalence and low titers (1:80), the presence of *Babesia microti* in Bridgeport is doubtful, because there is twofold variability in the reproducibility of antibody test results. Active infection by both the HGE agent and *B. burgdorferi* was indicated in many mice by the presence of ehrlichial DNA and cultivable spirochetes. The presence of the HGE agent and *B. burgdorferi* was confirmed for some of these animals by isolation in culture. In this study, *B. burgdorferi* was cultured from five of eight mice in April 1997. Similarly, the proportion of mice captured on 4 April 1998 with cultivable ehrlichiae was high (three of five). Previously, the HGE agent had been cultivated in HL-60 cells from murine blood of C3H/HeJ mice, which were infected by allowing HGE agent-infected *I. scapularis* ticks to engorge to repletion (28). Presence of ehrlichial DNA in mice in early April before nymphal *I. scapularis* are active suggests persistent infection in some individuals.

By contrast, however, many *P. leucopus* mice appear to have transient bacteremia. Many serum samples (37.5% of 80 mice) from the mice were serologically positive but PCR negative, and mice were just as likely to change from PCR positive to negative as PCR negative to positive. Few mice (*n* = 4) were PCR positive at every capture. The cell-mediated or humoral response may have been sufficient to decrease detection of the pathogen by PCR analyses and, consequently, ehrlichiae may be present in blood for short periods. Sera from these mice showed strong reactivity to many HGE proteins in immunoblots, especially the 44-kDa peptide (20). In an earlier study

(29), patency of the HGE agent was reported to be transient in most infected *P. leucopus* mice, lasting about 10 days, based upon blood smears, and a role for the humoral response was supported by chronic patent infection in splenectomized or B-cell-deficient mice. Infection by the HGE agent in PCR-negative and antibody-positive mice may be confined to the spleen or bone marrow or bacteremia may be below the threshold of the PCR assay. While *P. leucopus* may serve as a reservoir for the HGE agent, many of the mice in this study were evidently not infective or may have been only transiently infective, impacting both the prevalence of infection and degree of coinfection with *B. burgdorferi* in host-seeking ticks. Most of the larval pools were negative for the DNA of granulocytic ehrlichiae.

Nevertheless, the detection of ehrlichial DNA in larval *I. scapularis* taken from two wild white-footed mice with positive blood further supports the hypothesis that *P. leucopus* can be a reservoir for the HGE agent. Similarly, larval *I. scapularis* which fed on trapped, *Ehrlichia*-infected white-footed mice in the laboratory became infected (29). Xenodiagnostic *I. scapularis* larvae fed on laboratory mice infected with the HGE agent became infected, maintained infection, and transmitted the pathogen to uninfected mice, although the acquisition and transmission of the ehrlichial agent were less extensive than would be expected with *B. burgdorferi* (9). The ehrlichia-positive larval ticks removed from the PCR-negative mouse may have acquired the pathogen from the tissue as a nymph was in the process of infecting the mouse. One of the eight cofeeding nymphs from this mouse was strongly positive for the DNA of the HGE agent. Within 11 days from the time the nymph was removed, the mouse also showed a fourfold rise in antibody titer to BDS antigen. Pathogen transmission between cofeeding infected and uninfected ticks in the absence of a systemic infection in the host has been reported for several tick-borne viruses and *B. burgdorferi* (reviewed by Randolph et al. [25]). Whether this can occur with *Ehrlichia* is unknown. Alternatively, bacteremia may have been below the level of sensitivity of the PCR assay.

The prevalence of infection with the HGE agent in host-seeking *I. scapularis* in Connecticut and New York can be high and closely matches that for *B. burgdorferi*, suggesting, at least occasionally, a similar intensity of transmission. In Connecticut, 50% of adult and nymphal ticks had the DNA of the HGE agent (21), while 53% of the adult ticks and 21% of the nymphal ticks in Westchester County, N.Y., were determined by PCR to be infected (26). By contrast, the rate of coinfection in *I. scapularis* with *B. burgdorferi* and the HGE agent appears low (4 to 5%) in the nymphs (7, 26, 29). A higher prevalence of coinfection was reported from ticks collected from vegetation than from those collected from *P. leucopus* (13). The low prevalence of coinfection in *I. scapularis* with *B. burgdorferi* and the agent of HGE and dissimilarities in the infection rates between nymphs and adult ticks may indicate that these pathogens are not maintained or amplified by the same reservoir hosts (13, 26). Low coinfection in mouse-derived ticks may be, in part, a function of the transient infectivity of *Ehrlichia* in the mouse reservoir. Many white-footed mice may overcome infection but may be reinfected, possibly multiple times, through the season. Tick coinfection could derive from mice that are frequently reinfected or from a few persistently infected animals.

While the variation of prevalence of granulocytic ehrlichiae in tick populations at different locations and stages of the tick may reflect changes in infection in the mouse reservoir, these bacteria could be maintained in other reservoir hosts, such as eastern chipmunks (*Tamias striatus*) or white-tailed deer

(*Odocoileus virginianus*). White-tailed deer appear to be the reservoir for *E. chaffeensis*, the agent of HME, but a role for deer as reservoirs for the agent of HGE is undetermined. Using the 16S rDNA primers for granulocytic ehrlichiae, ehrlichial DNA was detected in blood samples from 18% of 63 deer from Connecticut, and 37% of these samples were also positive for the DNA of the 44-kDa gene of the HGE agent (19). Sequence analysis of the PCR products from two deer blood samples revealed that the DNA was identical to the p-44 gene sequence. Similarly, ehrlichial DNA was detected by PCR in 64% of 42 deer blood samples and 9.4% of 32 deer blood samples in Wisconsin and Maryland, respectively (6, 22). Most of the serum samples from Connecticut deer in 1996 (64% of 69 samples) contained antibodies to either or both of the BDS or NCH-1 strains of granulocytic ehrlichiae (19). Seroprevalence (60% of 294 samples) for antibodies to the HGE agent for Wisconsin white-tailed deer (31) was similar to that for Connecticut. Laboratory studies are needed to examine the perpetuation, reactivation, or reinfection of the HGE agent in *P. leucopus*. Further studies on the temporal changes in the reservoir competency of this mouse for granulocytic ehrlichiae, the interaction of multiple tick-borne pathogens in this animal, and the possible role of other vertebrates as reservoirs will help characterize the ecology and transmission of this newly emerging disease.

## ACKNOWLEDGMENTS

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