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## Hemocytic Rickettsia-Like Organisms in Ticks: Serologic Reactivity with Antisera to Ehrlichiae and Detection of DNA of Agent of Human Granulocytic Ehrlichiosis by PCR†

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**Ixodid ticks were collected from Connecticut, Massachusetts, Missouri, Pennsylvania, Rhode Island, and British Columbia (Canada) during 1991 to 1994 to determine the prevalence of infection with hemocytic (blood cell), rickettsia-like organisms. Hemolymph obtained from these ticks was analyzed by direct and indirect fluorescent antibody (FA) staining methods with dog, horse, or human sera containing antibodies to *Ehrlichia canis*, *Ehrlichia equi*, or *Rickettsia rickettsii*. Of the 693 nymphal and adult *Amblyomma americanum*, *Dermacentor variabilis*, *Ixodes scapularis*, and *Ixodes pacificus* ticks tested with dog anti-*E. canis* antiserum, 209 (32.5%) contained hemocytic bacteria. The prevalence of infected ticks varied greatly with species and locale. In parallel tests of duplicate hemolymph preparations from adult *I. scapularis* ticks, the hemocytic organisms reacted positively with *E. canis* and/or *E. equi* antisera, including sera from persons who had granulocytic ehrlichiosis. In separate PCR analyses, DNA of the agent of human granulocytic ehrlichiosis was detected in 59 (50.0%) of 118 adult and in 1 of 2 nymphal *I. scapularis* ticks tested from Connecticut. There was no evidence of *Ehrlichia chaffeensis* DNA in these ticks. In indirect FA tests of hemolymph for spotted fever group rickettsiae, the overall prevalence of infection was less than 4%. Specificity tests of antigens and antisera used in these studies revealed no cross-reactivity between *E. canis* and *E. equi* or between any of the ehrlichial reagents and those of *R. rickettsii*. The geographic distribution of hemocytic microorganisms with shared antigens to *Ehrlichia* species or spotted fever group rickettsiae is widespread.**

There is increased public awareness of tick-associated diseases in temperate regions of North America and Europe, and consequently, there is greater concern over tick bites. Although new diagnostic tests have aided clinical diagnoses, there remain unexplained febrile illnesses in persons who had been bitten by ticks. Historically, Rocky Mountain spotted fever, Colorado tick fever, tularemia, tick-borne relapsing fevers, and Texas cattle fever were among the most frequently studied diseases. More recently, human babesiosis, Lyme borreliosis, and ehrlichiosis have been recognized as new disease entities. The last was once considered to be primarily a veterinary problem, but the documentation of Sennetsu fever, isolation of *Ehrlichia chaffeensis* from human beings, and recent reports of human granulocytic ehrlichiosis clearly demonstrate a much broader scope and epidemiological importance (1, 2, 8, 13, 17, 22, 32, 33, 36).

In North America, human ehrlichiosis occurs in the southern and upper midwestern United States. *Amblyomma americanum* (lone-star tick) is the suspected vector of *E. chaffeensis* in southern states (2). Experimental transmission of this organism to white-tailed deer (*Odocoileus virginianus*) has been demonstrated (16). The causative agent of human granulocytic ehrlichiosis is believed to be *Ehrlichia equi* or a closely related

organism (8, 13). The modes of transmission for this agent and for *Ehrlichia risticii* (etiologic agent of Potomac horse fever), *Ehrlichia ewingii*, and *Ehrlichia platys* are unknown (36, 39), but ixodid (hard-bodied) ticks are suspected vectors. For example, in Minnesota and Wisconsin, where human granulocytic ehrlichiosis occurs (8, 13), patients recalled being bitten by *Ixodes scapularis* (formerly *I. dammini*) and *Dermacentor variabilis* (American dog tick). The former is closely related to *Ixodes ricinus*, a tick found in Europe that transmits *Ehrlichia phagocytophila*—the causative agent of tick-borne fever in sheep, cattle, and deer (18, 32).

Unidentified, rickettsia-like organisms have been observed in the hemocytes (blood cells) of *I. scapularis* and *D. variabilis* (31). These bacteria were detected by direct fluorescent antibody (FA) staining techniques utilizing dog anti-*Ehrlichia canis* antiserum. Use of a genus-specific monoclonal antibody by indirect FA staining methods reaffirmed that the hemocytic organisms shared antigens with *Ehrlichia* species. However, there was no reactivity of the unidentified organisms with human sera containing antibodies to *Rickettsia rickettsii*, the infectious agent of Rocky Mountain spotted fever. By utilizing FA staining and PCR methods, this study was conducted to determine (i) if there is widespread geographic distribution of hard-bodied ticks harboring hemocytic, rickettsia-like organisms, (ii) if the unidentified organisms in tick blood cells react with equid antisera to *E. equi* and with convalescent-phase sera from persons who had granulocytic ehrlichiosis, (iii) if DNA from the human granulocytic ehrlichiosis agent or *E. chaffeensis* is present in *I. scapularis*, and (iv) if tick blood cells also carry spotted fever group (SFG) rickettsiae.

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## MATERIALS AND METHODS

**Tick collection.** Adults of four tick species were collected by dragging flannel cloth over vegetation or by removing crawling ticks from persons' clothing in Vancouver Island, British Columbia (Canada), and in Connecticut, Massachusetts, Missouri, Rhode Island, and Pennsylvania during the period 1991 to 1994. *Ixodes pacificus* males and females were from Vancouver, British Columbia. Adult *I. scapularis* ticks were obtained from Bridgeport, East Haddam, Groton, Lyme, and Old Lyme, Connecticut; Nantucket, Massachusetts; Exeter, Prudence Island, and Block Island, Rhode Island; and Bryn Athyn and Erie (Presque Isle State Park), Pennsylvania. In addition, *A. americanum* nymphs and adults were collected from Missouri (Bollinger, Wayne, and Texas Counties) and from Prudence Island, Rhode Island. *D. variabilis* males and females also were obtained from Prudence Island, East Haddam, and Lyme. All unengorged ticks were kept alive until tested by being placed in a moist environment, such as in a plastic vial with a few blades of grass. The collection sites in Connecticut, Massachusetts, and Rhode Island are heavily infested with *I. scapularis*, and Lyme borreliosis is endemic, as indicated by the occurrence of human cases of infection (12, 38) and/or isolations of *Borrelia burgdorferi* (4–6) from ticks or white-footed mice (*Peromyscus leucopus*). Infected *I. scapularis* ticks and humans with Lyme borreliosis also have been reported from Bryn Athyn (3). In Missouri, *I. scapularis* and *A. americanum* are abundant. Human infections with *B. burgdorferi* are suspected there, but confirmatory evidence (i.e., isolation of *B. burgdorferi* from patients) is lacking. Although very little is known about the occurrence of human ehrlichiosis in or near any of the sampling areas in this study, there is evidence of canine (*E. canis*) and equine (*E. risticii*) ehrlichiosis coexisting with Lyme borreliosis in Connecticut (27, 35).

**FA staining.** Hemolymph was obtained from amputated tick legs (10) and placed singly or in duplicate on glass microscope slides. All preparations were air dried (incubated) overnight at 37°C. Briefly, cells were fixed in acetone, and fluorescein isothiocyanate (FITC)-conjugated dog anti-*E. canis* antibodies were applied. Infection was determined by direct FA staining methods (31). Also, an equid anti-*E. equi* antiserum used previously (8) was included for testing other preparations by indirect FA staining methods. Antibodies were raised in a horse experimentally infected with *E. equi* (MRK strain) and were kindly provided by J. Madigan, University of California, Davis. Serum was diluted to 1:80 in phosphate-buffered saline (PBS) solution (pH 7.2) and applied to tick cell preparations. After incubation for 30 min and washing, polyvalent FITC-labeled goat anti-horse immunoglobulin G antibodies (specific for heavy and light chains; Cooper Biomedical, Malvern, Pa.) were diluted in PBS solution to 1:40, applied to the slides, incubated, and washed before examination by fluorescence microscopy. For comparison, human serum samples from two persons who had granulocytic ehrlichiosis (8) were included in the analyses. By indirect FA staining methods with *E. equi* antigen, titration endpoints of 1:5,120 and 1:20,480 were recorded when a 1:40 dilution of conjugated goat anti-human immunoglobulin G antibodies (specific for heavy and light chains; Grand Island Biological Company, Grand Island, N.Y.) was used in the assays. In addition, serum from a person who had Rocky Mountain spotted fever (homologous antibody titer to *R. rickettsii*, 1:5,120) was diluted to 1:80 in PBS solution and used to determine if ticks harboring hemocytic organisms, which were reactive to antiehrlichial antisera, also carried SFG rickettsiae. Details about the source and testing of this serum specimen have been reported previously (29). All analyses included positive and normal serum controls used in previous work (28, 29, 31).

**Specificity tests for ehrlichiae.** The dog, equid, and human sera with antibodies to ehrlichiae, used to detect hemocytic rickettsia-like organisms in ticks, were tested against homologous and heterologous rickettsial antigens at The Connecticut Agricultural Experiment Station to assess specificity. The following antigens were tested with antisera by indirect FA staining methods: *E. canis*, *E. chaffeensis*, *E. equi*, and *E. risticii*. The sources and preparations of *E. canis* (causative agent of canine ehrlichiosis) and *E. risticii* antigens and homologous antisera have been described previously (27, 31). Antigen (DH 82 cells) and human sera containing antibodies to *E. chaffeensis* (Arkansas strain) were kindly provided by J. E. Dawson and J. G. Olson of the Centers for Disease Control and Prevention (Atlanta, Ga.). Details about the sources and use of *E. equi* reagents and specificity test results for analyses of spotted-fever antigens and sera have been reported previously (8, 29, 31).

**PCR analyses.** Adult *I. scapularis* ticks, collected in Bridgeport, East Haddam, and three sites in Lyme, Connecticut, during the fall of 1994, were analyzed for *E. chaffeensis* DNA and DNA from the agent of human granulocytic ehrlichiosis. Prior to testing, the ticks were kept in plastic vials over water in a closed plastic chamber at 7°C for 6 months.

DNA analyses were performed at the University of Maryland. Each tick was individually processed by bisection with a sterile blade to expose the internal tissues. The ticks were then placed into microcentrifuge tubes and triturated with tissue grinders in 30 µl of Tris-EDTA buffer (10 mM Tris, 1 mM EDTA [pH 8.5]). After complete disruption of the tick, Tween 20 (Sigma Chemical Co., St. Louis, Mo.) and proteinase K (Sigma) were added to obtain final concentrations of 1% and 200 µg/ml, respectively. The samples were then incubated for at least 3 h at 37°C, and proteinase K was inactivated by being heated at 100°C for 10 min. The samples were centrifuged to pellet the residual insoluble chitinous material, and the supernatant was used directly in all PCR analyses.

Each tick lysate was tested first for the presence of tick mitochondrial 16S

TABLE 1. Examination of host-seeking ticks for hemocytic, rickettsia-like organisms in British Columbia, Connecticut, Missouri, Rhode Island, and Pennsylvania during 1991 and 1992

| Tick species         | Life stage | Collection site (state or province) | Presence of rickettsia-like organisms <sup>a</sup> |                  |
|----------------------|------------|-------------------------------------|--|------------------|
|                      |            |                                     | Total no. of ticks tested                          | No. (%) positive |
| <i>A. americanum</i> | Nymph      | R.I.                                | 10   | 4 (40)           |
|                      | Male       | R.I.                                | 11   | 3 (27.3)         |
|                      | Female     | R.I.                                | 10   | 2 (20)           |
|                      | Male       | Mo.                                 | 29   | 24 (82.8)        |
|                      | Female     | Mo.                                 | 34   | 24 (70.6)        |
| <i>D. variabilis</i> | Male       | R.I.                                | 9  | 1 (11.1)         |
|                      | Female     | R.I.                                | 14   | 2 (14.3)         |
|                      | Male       | Conn.                               | 24   | 2 (8.3)          |
|                      | Female     | Conn.                               | 44   | 4 (9.1)          |
| <i>I. pacificus</i>  | Female     | B.C. <sup>b</sup>                   | 20   | 7 (35)           |
| <i>I. scapularis</i> | Nymph      | R.I.                                | 98   | 17 (17.4)        |
|                      | Male       | R.I.                                | 46   | 5 (10.9)         |
|                      | Female     | R.I.                                | 28   | 2 (7.1)          |
|                      | Male       | Conn.                               | 77   | 21 (27.3)        |
|                      | Female     | Conn.                               | 75   | 39 (52)          |
|                      | Male       | Pa.                                 | 40   | 7 (17.5)         |
| Female               | Pa.        | 74                                  | 45 (60.8)  |                  |

<sup>a</sup> Positive by application of FITC-conjugated dog anti-*E. canis* antiserum (prepared at the University of Illinois) to tick hemocytes.

<sup>b</sup> B.C., British Columbia.

rRNA genes as an amplification control (9). Samples in which tick 16S rRNA genes were successfully amplified were then subjected to PCR amplification of ehrlichial 16S rRNA gene sequences by using oligonucleotide primers specific for *E. chaffeensis* (HE1 and HE3) and the agent of human granulocytic ehrlichiosis (ge9f and ge10r) as described previously (2, 13). Amplified samples were analyzed by agarose gel electrophoresis and visualized with ethidium bromide. Samples that had no amplified products after the initial PCR were reamplified in a second PCR for maximum sensitivity. All sample preparations were performed in a pre-PCR laboratory separate from the laboratory in which the amplification and analysis were conducted. Each PCR included a water-only control and a positive control consisting of purified *E. chaffeensis* DNA or DNA obtained from a human patient with human granulocytic ehrlichiosis.

## RESULTS

Of the 643 nymphal and adult ticks tested in 1991 and 1992, 209 (32.5%) contained hemocytic, rickettsia-like microorganisms that reacted with the conjugated dog anti-*E. canis* antibodies by direct FA staining methods. Male and female *A. americanum*, *D. variabilis*, *I. pacificus*, and *I. scapularis* ticks harbored these organisms (Table 1). *A. americanum* and *I. scapularis* nymphs collected during June also had bacteria in their blood cells. The prevalence of infected ticks varied greatly, but the overall percentages of positive ticks were highest for *I. scapularis* (60.8% of 74 females) and *A. americanum* (82.8% of 29 males) ticks collected in Bryn Athyn, Pennsylvania, and in Missouri, respectively. In Bryn Athyn, 87.5% of 74 *I. scapularis* females were positive. In Lyme and Old Lyme, Connecticut, 6 of 8 male and 37.5% of 144 female *I. scapularis* ticks contained the hemocytic organisms during the spring and fall. As observed by fluorescence microscopy, many infected hemocytes contained numerous, uniformly sized granules within the cells.

During 1994, hemolymph was obtained from *I. scapularis* adults and tested with dog antiserum to *E. canis* and horse antiserum to *E. equi* by direct or indirect FA staining methods. As in 1991 and 1992, the number of infected females was highly

TABLE 2. Collection of *I. scapularis* females from Connecticut and Pennsylvania during October and November of 1994 and reactivity of hemocytic, rickettsia-like organisms with dog antiserum to *E. canis* and horse antiserum to *E. equi* by FA staining

| Sampling site            | Result with antiserum to:    |                  |                             |                  |
|--------------------------|------------------------------|------------------|-----------------------------|------------------|
|                          | <i>E. canis</i> <sup>a</sup> |                  | <i>E. equi</i> <sup>b</sup> |                  |
|                          | Total no. of females tested  | No. (%) positive | Total no. of females tested | No. (%) positive |
| Connecticut              |                              |                  |                             |                  |
| Bridgeport               | 59                           | 6 (10.2)         | 35                          | 4 (11.4)         |
| Groton                   | 27                           | 6 (22.2)         | 0                           |                  |
| Lyme                     | 82                           | 38 (46.3)        | 75                          | 9 (12)           |
| Pennsylvania, Bryn Athyn | 63                           | 19 (30.2)        | 60                          | 12 (20)          |
| Total                    | 231                          | 69 (29.9)        | 170                         | 25 (14.7)        |

<sup>a</sup> FITC-conjugated dog anti-*E. canis* antiserum used in direct FA staining methods.

<sup>b</sup> Horse anti-*E. equi* antiserum used in indirect FA staining methods. Note that some of the female ticks collected in Lyme and Bryn Athyn were tested for *E. canis* and *E. equi* by analysis of duplicate hemolymph preparations. There was additional parallel testing of hemolymph for SFG rickettsiae in ticks harboring rickettsia-like organisms that reacted positively with antisera to ehrlichiae. Females were chosen for tests because they yield more hemocytes.

variable. Prevalences of positive ticks ranged between 10.2 and 46.3% (Table 2). In general, the prevalence of ticks harboring hemocytic, rickettsia-like organisms was twofold greater (29.9% of 231) when dog antiserum to *E. canis* was used than when horse anti-*E. equi* antiserum was used (14.7% of 170). In analyses of duplicate hemolymph preparations from 48 *I. scapularis* ticks collected in Bryn Athyn, 3 (6.3%) ticks had hemocytic organisms that reacted with both antisera. In hemolymph samples from six other females, there was positive reactivity of hemocytic organisms only to the *E. canis* antiserum. The reverse was noted for 11 ticks (i.e., reactivity of hemocytic bacteria to *E. equi* antiserum). In the remaining 28 females, there was no reactivity to either antiserum. Similar findings were noted when duplicate hemolymph preparations from 70 female *I. scapularis* ticks collected in Lyme were tested. One female (1.4%) had bacteria in its hemocytes that reacted with both *E. canis* and *E. equi* antisera. Thirty-six (51.4%) females had hemocytic organisms that reacted positively when tested with *E. canis* antiserum but were nonreactive when tested with *E. equi* antiserum. The remaining hemolymph preparations from 33 ticks were negative, regardless of the antisera used. In analyses of duplicate hemolymph preparations from 33 male *I. scapularis* ticks collected from Bryn Athyn, hemocytic preparations from 3 of 21 ticks were positive when *E. canis* antiserum was used, while 1 of 12 males had hemocytic organisms that showed reactivity with horse anti-*E. equi* antiserum. There was no evidence of reactivity to both antiserum samples for hemolymph preparations from any of these male ticks.

Human serum samples from two persons who had granulocytic ehrlichiosis were analyzed in parallel tests with tick hemolymph preparations. In analyses of a second group of 48 *I. scapularis* females collected in Bryn Athyn, two (4.2%) had hemocytic organisms that reacted positively to one or the other serum sample and to the dog anti-*E. canis* antiserum. Hemocytes from 37 other females were positive to the dog antiserum but were negative when tested with the human sera. Hemolymph preparations from the remaining nine ticks were negative in both tests.

In separate PCR analyses of *I. scapularis* specimens collected from Bridgeport, East Haddam, and Lyme, Connecticut,

during November of 1994, 120 (88.9%) of 135 ticks had tick DNA that could be amplified. DNA of the human granulocytic ehrlichiosis agent was detected in 38 (45.2%) of 84 males, 21 (61.8%) of 34 females, and 1 of 2 nymphs analyzed. Positive ticks were collected from each site; the prevalences of infected ticks were 10.4% (48 tested) in Bridgeport, 73.8% (61 tested) in Lyme, and 90.9% (11 tested) in East Haddam. However, none of the 84 males, 34 females, and 2 nymphs tested for *E. chaffeensis* DNA were positive.

Duplicate hemolymph preparations were analyzed by FA staining methods to determine if hemocytes from *I. scapularis* contained rickettsia-like organisms that reacted to antiehrlichial antiserum or human sera with antibodies to *R. rickettsii*. In analyses of hemolymph from 70 females collected during 1994 from Bridgeport, Bryn Athyn, Groton, and Lyme, hemocytic bacteria reacted with antiserum to *E. canis* in 18 preparations. There was no reactivity in duplicate preparations when serum from a person who had Rocky Mountain spotted fever was tested. Hemocytes from four additional females were positive when anti-*E. equi* antiserum was included in the assays, but the hemocytic bacteria did not react with the anti-*R. rickettsii* antibodies in parallel tests. In separate analyses of ticks collected in 1994, there was no evidence of SFG rickettsiae in 67 male *I. scapularis* ticks collected from the four sites. These results were similar to those obtained in 1992 when no SFG rickettsiae were detected in *I. scapularis* ticks obtained in Connecticut and Pennsylvania (46 females and 21 males tested). A low prevalence of infection with SFG rickettsiae was noted for *A. americanum* ticks collected in Missouri (2 positive of 31 females tested and 1 positive of 21 males tested) and for *D. variabilis* ticks obtained in Connecticut (2 positive of 40 females tested and 1 positive of 19 males tested).

In specificity studies, antisera to various rickettsiae were tested against homologous and heterologous antigens. Homologous antibody titers ranged between 1:5,120 and 1:20,480 (Table 3). There was marked cross-reactivity when antisera to *E. canis* and *E. chaffeensis* were tested against the corresponding heterologous antigens. There was weak reactivity noted when a 1:80 dilution of *E. risticii* antiserum was screened against *E. canis* and *E. equi*. Negative results were recorded, however, when antisera to *E. canis* and *E. equi* were tested with *E. risticii* antigen. There was no cross-reactivity when *E. equi* antisera and antigens were screened in reciprocal tests against

TABLE 3. Reactivity of canine, equine, and human sera to *E. canis*, *E. chaffeensis*, *E. equi*, *E. risticii*, and *R. rickettsii* by indirect FA staining

| Antigen               | Reciprocal antibody titer <sup>a</sup> in serum samples |                                    |                             |                                 |                                   |
|-----------------------|---|------------------------------------|-----------------------------|---------------------------------|-----------------------------------|
|                       | <i>E. canis</i> <sup>b</sup>                            | <i>E. chaffeensis</i> <sup>c</sup> | <i>E. equi</i> <sup>d</sup> | <i>E. risticii</i> <sup>e</sup> | <i>R. rickettsii</i> <sup>f</sup> |
| <i>E. canis</i>       | 5,120   | 2,560                              | 0                           | 80                              | 0                                 |
| <i>E. chaffeensis</i> | 2,560   | 10,240                             | 0                           | 80                              | 0                                 |
| <i>E. equi</i>        | 0   | 0                                  | 20,480                      | 80                              | 0                                 |
| <i>E. risticii</i>    | 0   | 0                                  | 0                           | 5,120                           | 0                                 |
| <i>R. rickettsii</i>  | 0   | 0                                  | 0                           | 0                               | 5,120                             |

<sup>a</sup> Note that 0 indicates a titer of <80.

<sup>b</sup> Antiserum from a dog inoculated with *E. canis*, with a homologous titer determined previously (31).

<sup>c</sup> Serum from a person diagnosed with human monocytic ehrlichiosis (infected with *E. chaffeensis*).

<sup>d</sup> Antiserum from a horse inoculated with *E. equi* (MRK strain).

<sup>e</sup> Serum from a horse diagnosed with equine monocytic ehrlichiosis (Potomac horse fever); homologous titer determined previously (27).

<sup>f</sup> Serum from a person who had Rocky Mountain spotted fever (infected with *R. rickettsii*).

reagents for *E. canis* and *E. chaffeensis* or when heterologous tests included *R. rickettsii* antiserum and antigens. The FITC-conjugated dog anti-*E. canis* antibodies, used most frequently to detect hemocytic, rickettsia-like organisms in ticks, were strongly reactive with *E. canis* and *E. chaffeensis* antigens but were nonreactive with *E. equi* antigen. The *E. canis* antiserum did not detect purified preparations of *R. rickettsii* in controls.

## DISCUSSION

There is widespread geographic distribution of common, hard-bodied ticks carrying rickettsia-like organisms in their hemocytes. It is well established that ixodid ticks carry SFG rickettsiae (11, 21, 28–30). However, shared antigenicity of the unidentified, hemocytic organisms with ehrlichiae and highly variable frequency of occurrence in tick blood cells also are evident. Serologic tests indicating reactivity of hemocytic organisms with antisera to *E. equi* or the human granulocytic ehrlichiosis agent were confirmed when DNA from the latter was detected in tick lysates. We suspect that transovarial transmission of ehrlichiae may account, in part, for the high prevalences of infection. In contrast, no *I. scapularis* adults were infected with SFG rickettsiae, and the prevalence of *A. americanum* and *D. variabilis* ticks infected with these organisms was low. These results are consistent with those reported earlier (11, 28–30). On the basis of specificity studies of antisera used in our study, it appears that the unidentified bacteria most frequently observed in hemocytes of *A. americanum*, *D. variabilis*, *I. pacificus*, and *I. scapularis* were antigenically more closely related to ehrlichiae than to SFG rickettsiae. Moreover, we suspect that the hemocytic organisms may comprise at least two different ehrlichial species: one belonging to the *E. canis-E. chaffeensis-E. ewingii* group and the other being *E. equi*, the agent of human granulocytic ehrlichiosis, *E. phagocytophila*, or a closely related organism. Analysis of the 16S rRNA gene sequence of a human granulocytic ehrlichia, thought to be *E. equi* or a closely related agent, showed notable divergence between *E. equi* and *E. canis* or *E. chaffeensis* (13). These findings are supported by the present serologic test results and earlier observations (8, 14, 34). Antiserum to *E. equi* does not recognize *E. canis* and *E. chaffeensis* antigens and, at best, was weakly reactive with *E. risticii* by indirect FA staining methods. Although not known to occur in the continental United States, *Cowdria ruminantium*, a rickettsia classified within the tribe *Ehrlichieae* (36), might be present. Therefore, this organism should be considered along with other ehrlichiae in future serologic and epidemiologic studies.

Human and horse sera with antibodies to *E. equi* and dog antiserum to *E. canis* reacted with the hemocytic, rickettsia-like organisms when tested by FA staining methods. Canine and equine monocytic ehrlichioses occur in Connecticut, as indicated by clinical and serological data (27, 35). Human cases of infection with granulocytic ehrlichiosis have been reported in Wisconsin and Minnesota (8, 13), and there is documentation of human infections with *E. chaffeensis*, an agent closely related to *E. canis*, in the southern United States (1, 2). The lone-star tick is a suspected vector of *E. chaffeensis* (2, 16), while *I. scapularis* and *D. variabilis* may be involved in the transmission of *E. equi* or a closely related *Ehrlichia* species (13). In other studies, *A. americanum* transmitted *E. ewingii* (7). Our serologic and PCR results add further evidence that these and other tick species are probably carrying ehrlichiae and may be important vectors. However, isolation of these pathogens from ticks and persons (e.g., those who have granulocytic ehrlichiosis) is needed to substantiate more direct

vector-host relationships for these agents. Similar to studies conducted with *Rhipicephalus sanguineus* (brown dog tick) and *E. canis* (15, 19, 25) and *A. americanum* and *E. chaffeensis* (16), laboratory experiments are ultimately required to prove that *I. scapularis*, *I. pacificus*, or *D. variabilis* transmits *E. chaffeensis*, *E. equi*, the human granulocytic ehrlichiosis agent, *E. risticii*, or other ehrlichiae to mammals.

Ticks harbor rickettsia-like organisms in their hemocytes throughout most of the year. When in ticks, *E. canis* multiplies in hemocytes, midgut cells, and salivary glands (37). Horses are possible reservoirs of *E. equi* and *E. risticii* (26), and dogs infected with *E. canis* can pass this pathogen to attached ticks (19). In addition to adults feeding on large mammalian hosts, immature stages of *I. scapularis*, *I. pacificus*, and *D. variabilis* parasitize wild rodents (24). An agent in the tribe *Ehrlichieae* has been isolated from the spleen of a wild mouse, *Eothenomys kageus*, in Japan (23). Therefore, until further tests are conducted, rodent hosts as well as other forest-dwelling and domesticated mammals should be considered possible reservoirs of ehrlichiae.

With the reversion of agricultural land to forests over several decades, white-footed mouse and white-tailed deer populations have increased, particularly in the northeastern and upper midwestern United States. Consequently, *I. scapularis* and *D. variabilis* have likewise become more abundant in or near forests. The former tick species has a broader host range that includes birds (5, 24) and is a vector of *B. burgdorferi* and *Babesia microti* to human beings (6, 20, 24). However, *I. scapularis* does not seem to be as important as *D. variabilis* is in the etiology of Rocky Mountain spotted fever (28). Nonetheless, both tick species are abundant in rural and suburban environments and account for most of the tick bites encountered by persons living in these areas. The lone-star tick is not known to occur in Wisconsin and Minnesota and is considered rare in Connecticut. Because of the difficulty in isolating ehrlichiae from ticks in cell culture, we suggest that tick hemolymph, salivary gland tissues, and ovarian tissues be analyzed by PCR and DNA detection techniques to further document the presence of the human granulocytic ehrlichiosis agent or other *Ehrlichia* species and, along with serologic tests, to determine the prevalence of infection.

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