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INFECTIONS OF GRANULOCYTIC EHRLICHIAE AND *BORRELIA BURGDORFERI* IN WHITE-TAILED DEER IN CONNECTICUT

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ABSTRACT: Serum or whole blood samples, obtained from 141 white-tailed deer (*Odocoileus virginianus*) in Connecticut (USA) during 1980, 1991, and 1996, were analyzed to detect past or current infections of *Ehrlichia phagocytophila* genogroup organisms and *Borrelia burgdorferi*. When the BDS or NCH-1 strains of granulocytic ehrlichiae were used separately in indirect fluorescent antibody (IFA) staining methods, antibody positivity rates varied from 25 to 64% in 1991 and 1996, respectively. All 50 sera tested from 1980 collections were negative. Although percentages of sera with *B. burgdorferi* antibodies, as detected by an enzyme-linked immunosorbent assay, also differed (23 to 53%), there were coexisting antibodies to both bacteria in 20 (49%) of 41 sera. In tests on specificity, 19 deer sera with ehrlichial antibodies also were tested by IFA staining procedures for *Anaplasma marginale* antibodies; one serum with a titer of 1:5,120 to ehrlichial antigen reacted to *A. marginale* antigen at a serum dilution of 1:320. In parallel analyses of 69 sera, results of Western blot analyses for ehrlichial infections in deer were concordant (72% agreement) with those of IFA staining methods containing ehrlichial antigen. All positive immunoblots showed bands to peptides of the NCH-1 strain of the human granulocytic ehrlichiosis (HGE) agent having molecular masses of about 44, 105, or 110 kDa. In polymerase chain reaction (PCR) studies of blood samples from 63 deer, 11(18%) specimens were positive for 16S ribosomal DNA of an *Ehrlichia phagocytophila* genogroup organism, whereas 23 (37%) samples were positive for the DNA of the 44 kDa gene of the HGE agent. White-tailed deer are exposed to different tick-borne bacteria in areas where *Ixodes scapularis* ticks are abundant and may, in some instances, have had concurrent infections.

Key words: Borrelia burgdorferi, Ehrlichia phagocytophila genogroup, granulocytic ehrlichiosis, *Odocoileus virginianus,* serosurvey, white-tailed deer.

INTRODUCTION

Tick-associated illnesses, Lyme borreliosis, human babesiosis, and human granulocytic ehrlichiosis (HGE), have emerged as public health problems in or near forested areas of northeastern and upper midwestern United States where white-tailed deer (*Odocoileus virginianus*) and *Ixodes scapularis* ticks are abundant (Walker et al., 1996). Unlike white-footed mice (*Peromyscus leucopus*), deer are not known to be efficient reservoirs for *Borrelia burgdorferi* or *Babesia microti*. The role of deer in human or equine granulocytic ehrlichioses, caused by *Ehrlichia equi* or a closely related agent, is unknown. Based on 16S ribosomal DNA gene analyses, *E. equi,* the HGE agent, and *Ehrlichia phagocytophila* (the agent of tick-borne fever in cattle and sheep in Europe) are nearly identical (Walker and Dumler, 1996) and are classified as members of the *E. phagocytophila* genogroup. A granulocytic ehrlichial agent infects deer in Wisconsin (USA) and Georgia (USA) where *I. scapularis* is abundant (Belongia et al., 1997; Little et al., 1998), findings which support earlier work demonstrating DNA of the HGE agent in this tick in Wisconsin (Pancholi et al., 1995) and in northeastern United States (Magnarelli et al., 1995a; Telford et al., 1996; Schwartz et al., 1997; Yeh et al., 1997). The purpose of the present study was to detect antibodies to and DNA of *E. phagocytophila* genogroup organisms in deer sera and whole blood, respectively, and to determine seropositivity rates for possible coexisting infections with *B. burgdorferi* in sites where *I. scapularis* is abundant.

MATERIALS AND METHODS

Duplicate blood samples were collected at official state check stations from the body cavities of each of 69 deer killed in 11 towns during the fall hunting season (November and December) of 1996. Sera were obtained from blood samples following centrifugation at 2,000× *g* for 30 min. and were stored at −60 C until antibody analyses could be performed. The second blood sample contained EDTA and was used for polymerase chain reaction (PCR) studies. Nearly all of the animals were from heavily tick-infested areas of Middlesex and New London Counties in southern Connecticut (USA), regions where human cases of Lyme borreliosis, babesiosis and granulocytic ehrlichiosis occur. Additional sera, taken from archived collections at the Connecticut Agricultural Experiment Station, were available from deer in northwestern Connecticut (Sharon) where *I. scapularis* is far less abundant (Magnarelli et al., 1993).

Seventy-two deer sera, obtained in 1980 and 1991 were selected from stored collections kept at −60 C at the Connecticut Agricultural Experiment Station. There were representative specimens from four towns in Middlesex and New London Counties in southeastern Connecticut and from deer killed in Fairfield County (southwestern Connecticut) and Litchfield County (northwestern Connecticut). Collectively, sera were from deer killed in a broad region of Connecticut extending from Sharon (41°52′N, 72°26′W) to Montville (41°27′N, 72°10′W). Details on the collection of these sera have been reported (Magnarelli et al., 1991, 1993).

An enzyme-linked immunosorbent assay (ELISA) or indirect fluorescent antibody (IFA) staining methods were used to detect total antibodies to strain 2591 of *B. burgdorferi* (Magnarelli et al., 1991, 1993). In analyses for ehrlichial antibody, antigen was derived from either of two strains of granulocytic ehrlichiae (BDS and NCH-1). The BDS strain is a human isolate from Minnesota (USA) that had been inoculated into horses. Infected horse neutrophils, fixed to glass microscope slides, were purchased from J. Madigan of the University of California (Davis, California, USA). The NCH-1 strain, originally isolated from a human in Nantucket, Massachusetts (USA; Telford et al., 1996), was cultured in human promyelocytic leukemia (HL-60) cells at Yale University (IJdo et al., 1997). Deer sera were serially diluted in phosphate-buffered saline (PBS) solution (pH = 7.2) and tested for total antibodies with a 1:15 dilution of polyvalent fluorescein isothiocyanate (FITC) conjugated goat anti-deer immunoglobulin (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland USA). Reactivity of ehrlichial antigens was confirmed by testing positive sera from HGE patients, whereas the reactivity of the FITC-labeled anti-deer antibody reagent was verified by testing deer sera that had immunoglobulins to *B. burgdorferi*. Details on the sources of reagents, dilutions of conjugates, and on other aspects of performing IFA staining methods and determining titration end points have been reported (Magnarelli et al., 1993, 1995b). Evidence of antibody presence in positive control and test sera was based on conservative grading of distinct fluorescence of morulae (inclusion bodies) in infected neutrophils or HL-60 cells.

Tests on specificity were conducted to determine the lowest serum dilution that had no non-specific reactivity. Four deer sera from animals with no known exposure to tick-borne pathogens, were provided by P. Luttrell (Southeastern Cooperative Wildlife Disease Study, University of Georgia, Athens, Georgia, USA). There were 11 other sera from this source that contained antibodies to *B. burgdorferi* following inoculations of this bacterium. Reciprocal tests were performed on an additional 91 sera, collected in 1991 and 1996, to determine if antibodies to the Lyme borreliosis agent (n = 41 samples) or *E. phagocytophila* genogroup organisms (n = 50 samples) cross-reacted with heterologous antigens by IFA staining methods. Included in this group were 11 deer samples found to be positive for ehrlichial DNA. Although not known to be a common or persistent infection in deer in northeastern United States, *Anaplasma marginale* antigen, which consisted of infected red blood cells from cattle, was included in specificity trials as well as in separate tests of the 69 field-collected deer sera. The *Anaplasma* antigen and seven positive and six negative control cattle sera were provided by G. Wagner and S. Waghela (Texas A&M University, College Station, Texas, USA) and K. Kocan (Oklahoma State University, Stillwater, Oklahoma, USA). The same procedures used in IFA analyses for ehrlichial antibodies were applied in tests for *A. marginale* antibodies. In analyses of the cattle sera, FITC-labeled goat anti-bovine antibodies (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland, USA) were diluted to 1:60 in PBS solution and tested with sera diluted to 1:80.

Western blot analyses were performed to confirm results of IFA staining methods, to identify key banding patterns for granulocytic ehrlichial infections, and to determine if *A. marginale* antiserum reacted with the NCH-1 strain of the HGE agent. Sufficient amounts of sera were available for analyses of 135 of 141

deer samples collected during 1980, 1991 and 1996. Preparation and use of lysates of infected or uninfected (i.e., control) HL-60 cells, blocking and washing solutions, test sera, molecular mass standards, nitrocellulose sheets, and the use of a chemiluminescence detection system have been described (IJdo et al., 1997; Magnarelli et al., 1997a). Deer sera were diluted to 1:100 in PBS solution. The conjugate (alkaline phosphatase-labeled F(ab')2 anti-deer immunoglobulin was commercially prepared (Kirkegaard and Perry Laboratories) and diluted to 1:1,000 in PBS solution. All immunoblots contained positive (i.e., from those deer found to have DNA of the HGE agent in blood) and negative serum controls and parallel testing of each serum sample with infected and uninfected lysates of HL-60 cells. Multiple sera obtained from two cows before and after inoculation with *A. marginale* were diluted to 1:100 in PBS solution and included in Western blot analyses (Vidotto et al., 1994) to test the reactivity of antibodies to this agent with lysates of the NCH-1 strain and uninfected HL-60 cells. The immunoblotting methods used to detect antibodies to ehrlichiae were applied in analyses for *A. marginale* antibodies.

Whole deer blood samples containing EDTA were used to prepare genomic DNA for PCR analyses as directed with a Qiagen DNA extraction kit (Qiagen Inc., Valencia, California, USA). Two hundred μl of deer blood were incubated with 25 μl of protease K (10 mg/ml) and 200 μl of AL buffer as specified by the manufacturer (Quiagen, Inc.) at 70 C for 15 min. Then, 210 μl of 100% ethanol were added and the total volume was loaded on to a DNA extraction column and centrifuged at 6,000× *g* for 1 min. The column was washed twice with washing buffer, and the DNA was eluted from the column with 200 μl of sterile water at 70 C and stored at −20 C. Negative and positive samples were included with each DNA extraction procedure. Primers used for the PCR analyses included ehr 521 and ehr 747, which allow for amplification of a portion of the 16S ribosomal DNA gene (Pancholi et al., 1995). A second set of primers (5′-TCAAGACCAAGGGTATTAGAGATAG-3′ and 5′-GCCACTATGGTTTTTTCTTCGGG-3′) starting at positions 396 and 921, respectively, and based on the sequence of the hge-44 gene that encodes the immunodominant 44 kDa protein of the HGE agent, was used in parallel PCR analyses (IJdo et al., 1998). A third set of primers designed to amplify a portion of the major surface protein (msp)-2 gene of *A. marginale* (5′-TTCCCGTACAAGCGAGATGCTG-3′ and 5′-TCGTGTTGATGGTGGTATTGTCC-3′) starting at positions 241 and 763, respectively, (Palmer et al., 1994) was included in additional analyses. For all PCR procedures, known positive and negative samples were included. The denaturing, annealing and extension temperatures were 94, 55, and 72 C, respectively, for one min at each step for 30 cycles. The reaction contained 5μl PCR buffer (Boehringer, Mannheim, Germany), 0.25 μM primer (each), 0.2 μM dNTP, 2μl DNA template and 2.5 U of Taq polymerase (Boehringer); distilled water was added to a total volume of 50 μl. The specificity of the different PCR primer sets was assessed by using DNA samples from cultured NCH-1 and *A. marginale*-infected (about 71% infected red blood cells per ml) or non-infected whole cow blood provided by G. Wagner and S. Waghela. The PCR products from two deer blood samples, using the presumably more specific hge-44 primers, were sequenced in the W. M. Keck Foundation Biotechnology Resource Laboratory (Yale University, New Haven, Connecticut, USA) using Taq FS DNA polymerase and fluorescence-labeled dideoxynucleotides in a thermal cycling protocol. Both hge-44 primers were used to sequence both DNA strands to reduce the possibility of sequencing errors.

In 2 × 2 contingency table analyses, the Fisher's exact test was used to compare the number of positive serum samples collected in different years. Analyses were performed by using statistical software programs (SigmaStat, SPSS Inc., Chicago, Illinois, USA).

RESULTS

There was no serologic cross-reactivity when control sera containing antibodies to *B. burgdorferi*, *E. phagocytophila* genogroup organisms or *A. marginale* were tested by IFA staining methods with heterologous antigens at serum dilutions of ≥1:80. However, at serum dilutions of 1:20 or 1:40, weak fluorescence of antigens was observed, particularly when antibodies to *A. marginale* and granulocytic ehrlichiae were screened with the respective heterologous antigens. Based on this, the reactivity of deer sera to the BDS or NCH-1 strains of granulocytic ehrlichiae at serum dilutions of 1:80 or greater were considered positive. Homologous antibody titers of ≥1:1,280 were recorded in all other analyses of sera paired with each of the three pathogens. Results of Western blot analyses of seven sera from cattle with antibodies to *A. marginale* confirmed IFA

TABLE 1. Detection of total antibodies to *Borrelia burgdorferi* or to the BDS or NCH-1 strains of granulocytic ehrlichiae by enzyme-linked immunosorbent assay or indirect fluorescent antibody staining methods of deer sera obtained in Connecticut during 1980, 1991, and 1996.

Sampling year	Number of serum samples tested	Number and (%) with antibodies to		
		B. burgdorferi[a]	BDS strain[b]	NCH-1 strain[b]
1980[c]	50	0	0	0
1991[d]	22	5 (23)	6 (27)	14 (64)
1996[d]	69	36 (53)	17 (25)	19 (28)

[a] Tested by an enzyme-linked immunosorbent assay. Positive titer ≥1:160.

[b] Tested by indirect fluorescent antibody staining methods. Positive titer ≥1:80.

[c] Sera collected in northwestern Connecticut, an area where *I. scapularis* ticks and Lyme disease cases were uncommon in 1980.

[d] Sera collected statewide in areas where *I. scapularis* ticks were abundant.

findings by showing no reactivity with the NCH-1 strain of the HGE agent, while immunoblotting of these positive control sera for *A. marginale* reaffirmed the presence of homologous antibodies.

Deer sera collected in 1980 from northwestern Connecticut were negative to *B. burgdorferi* and granulocytic ehrlichiae, but sera obtained in 1991 and 1996 contained antibodies that reacted with both pathogens. Seropositivity rates were highly variable (Table 1). In IFA analyses for ehrlichial antibodies, seropositivity rates for the NCH-1 strain were greater than those calculated for the BDS strain. Results were concordant for 61(67%) of the 91 sera obtained in 1991 and 1996. During 1991, percentages of positive sera in IFA analyses with the NCH-1 strain of the HGE agent (64%) or the BDS strain (27%) were greater than the proportion of positive sera with *B. burgdorferi* antibodies (23%). In 1996, seropositivity for *B. burgdorferi* (53%) greatly exceeded values for granulocytic ehrlichiae (25 to 28%). There was no significant difference in the number of positive reactions recorded for *B. burgdorferi* or either ehrlichial strain when results for 1980 (i.e., by rows or columns) were compared with those recorded for 1996 ($P = 1.000$). Similarly, when results for the BDS and NCH-1 strains recorded in 1980 were compared with those of 1996, there was no significant difference ($P = 1.000$). Antibody titers ranged from 1:160 to 1:5,120 in an ELISA for *B. burgdorferi* infections and from 1:80 to 1:5,120 by IFA staining methods for granulocytic ehrlichial infections. Collectively, 20 (49%) of 41 sera with antibodies that reacted with *B. burgdorferi* also contained ehrlichial antibodies to either or both strains, while 23 of 25 sera without antibodies to *B. burgdorferi* had immunoglobulins that reacted with either or both ehrlichial strains.

Deer sera with or without antibody reactivity to the NCH-1 strain of the HGE agent were tested with *A. marginale* antigen by IFA staining methods. Of the 19 sera collected in 1996 and found to be positive for ehrlichial antibodies, one serum sample having an antibody titer of 1:5,120 to the NCH-1 strain also reacted with *A. marginale* antigen at a dilution of 1:320. A whole blood sample from this deer lacked ehrlichial DNA in PCR analyses with different primer sets. The remaining 18 sera with antibodies that reacted with the NCH-1 strain and 25 sera without antibodies reactive to ehrlichiae were negative in antibody tests with *A. marginale*.

Deer sera were analyzed by Western blot procedures to confirm results of IFA staining methods and to identify key banding patterns for ehrlichial infections. All 50 deer sera collected in northwestern Connecticut during 1980 were negative in both assays. Of the 69 sera collected in 1996 and tested by IFA and immunoblotting methods with the NCH-1 strain, findings were highly concordant (72%); 17 and 33 sera were positive or negative in both assays. Results for the remaining 19 sera (28%) differed. Of these, two samples were positive by IFA staining methods and negative in immunoblots, while the reverse was noted for 17 sera. There were

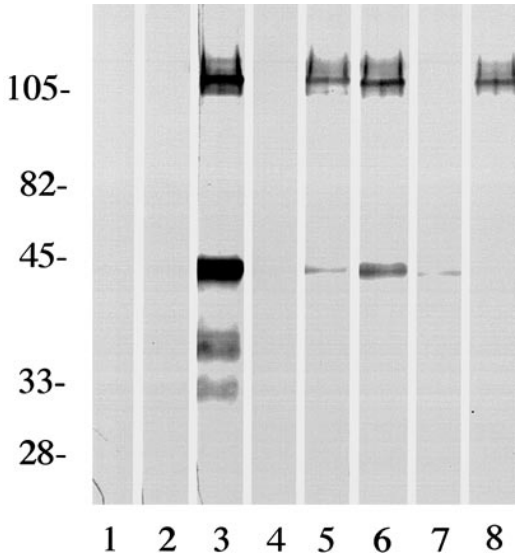

FIGURE 1. Representative immunoblots of individual deer sera collected in Connecticut during 1996 showing reactivities to lysates of the NCH-1 strain of the HGE agent. Molecular masses indicated in kilodaltons. Lanes 3, 5, 6 and 8 show reactivity of seropositive deer. Lane 3 shows reactivity of a deer serum sample having the highest antibody titer (1:5,120), as determined by indirect fluorescent antibody methods with the NCH-1 strain. Protein antigens having molecular masses of about 44 kDa, 105 kDa, or 110 kDa were reactive. Lanes 1, 2 and 4 show no reactivity of deer sera, while very weak reactivity to the 44 kDa protein in lane 7 was graded as a negative.

bands to peptides of the HGE agent having molecular masses of about 44, 105, or 110 kDa (Fig. 1). Distinct reactivity to the 44 kDa protein, singly (n = 9 sera) or in combination with the other peptides (n = 12 sera), occurred most frequently in 21 (62%) of 34 positive sera. In the other 13 sera, distinct bands to the 105 kDa protein were associated with very weak antibody reactivity to the 110 kDa peptide.

Results of PCR analyses for 63 available deer blood specimens were compared with those of IFA and Western blot serum analyses. Patterns of reactivity in DNA analyses differed, and discordance was noted when results of PCR and antibody analyses were compared. For example, 27 (43%) of 63 sera and blood specimens were positive (n = 6) or negative (n = 21) in both antibody assays with the NCH-1 strain and

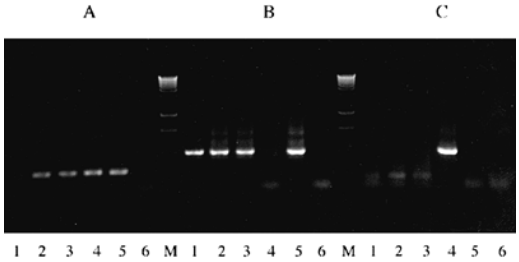

FIGURE 2. Reactivities of deer blood DNA samples (lanes 1-3), *Anaplasma marginale* DNA (lane 4), and human granulocytic ehrlichial DNA of the NCH-1 strain (lane 5) in PCR analyses with different primer sets (A = 16S primers ehr 521 and ehr 747; B = hge-44 primers 8F and 9B; and C = major surface protein-2 primers of *A. marginale*). PCR buffer solution served as a negative control (lane 6), while the molecular size marker (lane M) is the 1-kilobase ladder.

in PCR analyses which included the hge-44 primers. Results for the remaining 36 sera differed. There was greater sensitivity when the hge-44 primers were used (37% positive), compared to results of assays with the 16S ribosomal DNA primers (18%). Compared to PCR analyses, antibody detection assays of the companion serum samples were less variable and revealed evidence of ehrlichial infection in 32 (51%) and 33 (52%) samples, respectively.

Three different primer sets, used in PCR assays of deer blood samples, were assessed for specificity. Results for three deer blood samples collected in Hebron, Colchester, and Marlborough, Connecticut, one cow blood sample with *A. marginale* DNA, one cultured NCH-1 sample, and a negative control containing the reaction mixture without template are shown in Figure 2. The 16S ribosomal DNA primers produced a faintly visible product for the first deer sample as well as more definite products for the second and third deer samples, the *A. marginale* DNA, and the NCH-1 DNA sample (Fig. 2A). Using the hge-44 primers, only the three deer samples and the NCH-1 sample were positive (Fig. 2B), while the msp-2 primers yielded only a product in the *A. marginale* sample (Fig. 2C). Sequence analyses of the

PCR products from two deer blood samples, collected in Hebron and Colchester, revealed that the DNA was identical to the hge-44 gene sequence.

Deer samples containing antibodies to or the DNA of granulocytic ehrlichiae were from 21 sites in Connecticut. Although the majority of positive specimens were collected in tick-infested areas of southcentral and southeastern regions of the state, there was serologic evidence of granulocytic ehrlichial infections in the northern parts of Connecticut, including the towns of Sharon, Union, and Stafford. Ehrlichial DNA and antibodies were detected in deer blood samples from Colchester, East Haddam, East Hampton, Glastonbury, Hebron, and Marlborough.

DISCUSSION

Antibody test results and PCR data, including results of sequence analyses, indicated that white-tailed deer were exposed to *E. phagocytophila* genogroup organisms at widely separated sites in Connecticut where *I. scapularis* ticks abound. These findings correlate with the occurrence of HGE cases and equine granulocytic infections (Hardalo et al., 1995; Madigan et al., 1996; IJdo et al., 1997) and with results of DNA analyses demonstrating similar infections in *I. scapularis* (Magnarelli et al., 1995a). Utilizing the same group-specific primer set in PCR studies of deer as investigators in Wisconsin (Belongia et al., 1997), our results (18% positive) compared favorably with their findings (15% positive). However, our use of the hge-44 primers showed much greater sensitivity than assays with the 16S ribosomal DNA primers. Further, seropositivity rates for Connecticut deer, determined by IFA staining methods with different strains of antigen, were at least two-fold greater than that (8%) reported in Wisconsin. More recent findings (Walls et al., 1998), however, indicate seropositivity rates ranging from 47% to 60% for Wisconsin deer. In other studies, ehrlichial DNA was detected by PCR in 27 (64%) of 42 blood samples obtained from deer in the upper midwestern United States (Bakken et al., 1996) and in 3 (9%) of 32 deer blood specimens in Maryland (Massung et al., 1998). It is unknown whether or not deer serve as reservoirs for *E. phagocytophila* genogroup organisms, but they may prove useful in surveillance studies to identify endemic areas for granulocytic ehrlichiae.

Based on IFA staining results, antibodies reactive to *B. burgdorferi* and granulocytic ehrlichiae co-existed in deer sera collected in 1991 and 1996. There was no evidence of either infection in deer sera obtained in 1980 from northwestern Connecticut when *I. scapularis* was scarce then (Magnarelli et al., 1993). It is unclear if the seropositive deer in southern Connecticut had simultaneous infections, but with heavy parasitism by *I. scapularis* immatures and adults throughout most seasons, some concurrent infections are likely to occur. Since our test results on specificity indicate no cross-reactivity between *B. burgdorferi* and *E. phagocytophila* genogroup organisms, we conclude that our serologic findings indicate that deer were exposed to unrelated agents. These results are similar to those published for humans (Magnarelli et al., 1995b), dogs (Magnarelli et al., 1997a), and white-footed mice (Magnarelli et al., 1997b).

Further testing was necessary to assess possible cross-reactivity between *E. phagocytophila* genogroup organisms and *A. marginale.* The use of primers ehr 521 and ehr 747 amplified *A. marginale* DNA in our PCR analyses, which reaffirmed the high degree of sequence homology noted between this organism and the HGE agent (Van Vliet et al., 1992; Pancholi et al., 1995). Use of the hge-44 primers in our laboratory showed greater specificity. Our PCR assay with the msp-2 primers did not detect the DNA of *E. phagocytophila* genogroup organisms in three deer blood samples or the DNA of the NCH-1 strain but did indicate the presence of *A. marginale* DNA (positive control). Aside from

one serum sample, which had a high antibody titer (1:5,120) to the NCH-1 strain of the HGE agent and cross-reacted at a serum dilution of 1:320 with *A. marginale* antigen, our IFA staining methods revealed no serologic evidence of *A. marginale* infections in deer. Although this pathogen normally occurs in cattle and is not known to occur in northeastern United States, it reportedly infects deer and elk (*Cervus elaphus*) elsewhere (Keel et al., 1995; Zaugg et al., 1996). Therefore, the geographic distribution of *A. marginale* should be taken into consideration when the ehr primers are used in PCR assays to detect the DNA of ehrlichiae in the *E. phagocytophila* genogroup. We suggest that the more specific hge-44 primers be used whenever possible.

Results of Western blot analyses closely agreed with those obtained by IFA staining methods. Both procedures can be used to identify past or current ehrlichial infections in deer. Detection of antibodies to the 44 and 105 kDa proteins of the NCH-1 strain indicates that these proteins are key markers for infection. This finding is similar to published results for human (IJdo et al., 1997; Ravyn et al., 1998) and dog sera (Magnarelli et al., 1997a) containing antibodies to this strain of the HGE agent. The gene encoding the 44-kDa antigen has been cloned and expressed (IJdo et al., 1998; Zhi et al., 1998). The use of this highly specific recombinant antigen in an ELISA or Western blot analyses might improve serum antibody detection. Nonetheless, Western blot analysis with whole cell antigens is a suitable adjunct procedure that can be used along with IFA staining methods, even though fewer bands were present in analyses of deer sera.

As previously reported (Belongia et al., 1997), antibody test results and PCR findings usually differed. Onset of antibody production to *E. phagocytophila* genogroup organisms after initial infection and duration of antibody presence in deer are unknown. It is possible that ehrlichiae are present in blood for short periods (e.g., a few weeks) and that the deer had mounted cell-mediated or humoral immune responses sufficient to decrease direct detection of the pathogen by PCR analyses. In equine granulocytic ehrlichial infections, DNA detection methods were more useful in testing blood samples obtained during acute infection (Heimer et al., 1997) rather than in the analyses of specimens collected during the convalescent phase. Utilizing isolation techniques, it is important to determine if deer are acutely infected during a particular season (e.g., summer or fall). Challenge studies in deer also are needed to determine when pathogens and antibodies can first be detected post inoculation, if pathogenic effects are associated with granulocytic ehrlichial infections, the duration of pathogen (or DNA) and antibody persistence in blood, and to determine reservoir competency of deer.

The use of HL-60 cell cultures has facilitated the isolation of granulocytic ehrlichiae from humans (Goodman et al., 1996) and horses (Heimer et al., 1997). Organisms cultured from the blood of these hosts in northeastern and upper midwestern United States have been identified as being members of the *E. phagocytophila* genogroup. Another organism found in white-tailed deer in Georgia and Oklahoma (USA) (Dawson et al., 1996) appears to be closely related to members of this genogroup and *Ehrlichia platys,* but it is unclear if this same bacterium occurs in Connecticut. Our DNA sequencing results indicate that the ehrlichial organism in Connecticut deer is very similar to the agent that infects human beings. However, further studies are needed to determine if different strains of the HGE agent infect white-tailed deer in northeastern and northcentral United States.

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