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Serologic Evidence of Canine and Equine Ehrlichiosis in Northeastern United States

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In a retrospective study, indirect fluorescent-antibody staining methods were used to detect immunoglobulins to *Ehrlichia canis* and *Ehrlichia risticii* in canine and equine sera that had originally been analyzed for antibodies to *Borrelia burgdorferi*. Analyses of 60 dog serum specimens collected in Connecticut and New York State during 1986 revealed antibodies to *E. canis* in 7 (11.7%) specimens; titration endpoints ranged from 1:40 to 1:320. Three of these dogs had anemia. Of the 187 equine serum specimens obtained in Connecticut during 1985 and analyzed by indirect fluorescent-antibody staining methods, 17 (9.1%) contained antibodies to *E. risticii*. Maximal antibody titers of 1:1,280 were recorded for serum specimens collected from three equids during May and July. We conclude that canine and equine ehrlichiosis coexist with Lyme borreliosis in Connecticut and the lower Hudson River Valley of New York State.

Obligate intracellular rickettsiae in the tribe *Ehrlichieae* can cause disease in humans and domesticated animals. *Ehrlichia canis* and *Ehrlichia risticii*, the causative agents of canine ehrlichiosis and equine monocytic ehrlichiosis (Potomac horse fever), respectively, are significant veterinary pathogens. These microorganisms, like other *Ehrlichia* species, infect circulating leukocytes and other cells (6, 23, 27). Canine ehrlichiosis (tropical canine pancytopenia) was originally described in Algeria but is now recognized in the Middle East, the Orient, and widely separated areas of the United States (6, 7, 23). Equine monocytic ehrlichiosis, first discovered in Maryland and Virginia (8), also occurs in the northeastern United States and parts of Western Europe (22, 24). Ticks are known or suspected vectors of ehrlichiae. Although the brown dog tick, *Rhipicephalus sanguineus*, can efficiently transmit *E. canis* to dogs (3), the arthropod vector of *E. risticii* is unknown.

During the past three decades, populations of hard-bodied ticks have increased in the eastern United States. In New England, *Ixodes scapularis* (formerly known as *Ixodes dammini* [21]) is abundant in forested areas and transmits *Borrelia burgdorferi*, the etiologic agent of Lyme borreliosis, and *Babesia microti*, a protozoan parasite. American dog ticks, *Dermacentor variabilis*, also abound in or near woodlands and can transmit *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever. Antibodies to these pathogens have been detected in dogs, cats, equids, mice, and other mammals (1, 10–17). Having frequent contact with ticks, these hosts may acquire one or more pathogens. The purpose of the present study was to determine whether dogs and equids living in tick-infested areas of the northeastern United States were exposed to ehrlichiae.

MATERIALS AND METHODS

Source of serum specimens and clinical records. Blood samples were collected from dogs and equids by veterinarians during 1985 and 1986. The serum specimens were analyzed previously for antibodies to *B. burgdorferi* (10, 15–17) and were stored at −60°C at the Connecticut Agricultural Experiment Station. Serum specimens from 60 privately owned dogs, representing a variety of breeds, were selected for the present study. These dogs had unknown illnesses, were tentatively diagnosed as having Lyme borreliosis, and lived in tick-infested areas of Connecticut and in the lower Hudson River Valley region of New York State. As diagnosed by veterinarians, they presented with one or more of the following nonspecific signs: limb or joint disorders, fever, lethargy, anorexia, and lymphadenopathy. The most common signs were lameness (n = 34 dogs), fever (n = 24), anorexia (n = 23), and lethargy (n = 22). In most cases, information on hematologic findings and age also was available. History of tick bite or exposure was common for these dogs, but specific information on travel histories was unavailable. In addition to choosing serum specimens from ill dogs, an effort was made to select samples from a broad geographic area. The present study included serum specimens from dogs from 17 towns in Connecticut (Fairfield, Hartford, Middlesex, New Haven, and New London counties) and 24 communities in New York State (New York City, Dutchess, Westchester, and Putnam counties). Half of the serum samples were from dogs living in Westchester (n = 19) and Middlesex (n = 11) counties. Equine serum samples were from 187 animals living in 81 towns in Connecticut. Specimens from all eight counties were analyzed, but the majority of equids (69%) lived in Fairfield (n = 56), Litchfield (n = 19), Middlesex (n = 17), New Haven (n = 19), and Tolland (n = 18) counties. Clinical data and precise information on travel histories, however, for the equids were unavailable.

Serologic analyses. Indirect fluorescent-antibody (IFA) staining methods were used to detect antibodies to *E. canis* and *E. risticii*. In general, the procedures described earlier (25, 26) were followed. Antigens of *E. canis* and *E. risticii* (i.e., canine and equine infected monocytes) were obtained from the University of Illinois (Urbana) and the Naval Medical Research Institute (Bethesda, Md.). Polyvalent fluorescein isothiocyanate (FITC)-labeled rabbit anti-dog immunoglobulins (GIBCO Laboratories, Grand Island, N.Y.) and polyvalent FITC-conjugated goat anti-horse antibodies (specific for heavy and light chains of immunoglobulin G; Cooper Biomedical, Malvern, Pa.) were diluted in

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TABLE 1. Reactivities of serum specimens collected in 1986 from 60 dogs and tested by IFA staining methods for antibodies (total immunoglobulin) to *E. canis*

Sampling period	Connecticut			New York State		
	Total no. of serum specimens	No. positive	Antibody titer	Total no. of serum specimens	No. positive	Antibody titer
January–February	5	3	1:40	6	0	
March–April	4	1	1:160	7	0	
May–June	2	0		7	1	1:320
July–August	5	0		8	1	1:160
September–October	8	1	1:80	8	0	

phosphate-buffered saline solutions to 1:50 and 1:40, respectively, before use. Positive controls, also obtained from the University of Illinois, consisted of sera from a dog inoculated with *E. canis* and a horse clinically diagnosed with Potomac horse fever. Homologous antibody titers of ≥1:5,120 were recorded by IFA staining methods. Normal serum specimens were from dogs and equids with no history of ehrlichiosis. Serial dilutions of test sera were screened against *E. canis* and *E. risticii*, and the respective antibody titers of ≥1:20 and ≥1:80, as determined earlier (25, 26), were considered positive. Although the dog sera were analyzed against *E. canis* and the horse sera were screened against *E. risticii*, subsets of serum specimens (including positive and negative controls) were tested with heterologous and homologous ehrlichial antigens to check these cutoff values and to assess specificity and reproducibility. On the basis of specificity test results, the cutoff values for positive results determined by other investigators (25, 26) seemed appropriate. Also, IFA staining methods were used to determine whether antibodies to *B. burgdorferi* were present in positive and negative control sera for *Ehrlichia* species. Details on these serologic tests have been reported previously (16, 17).

RESULTS

Serologic tests detected antibodies to *E. canis* in 7 (11.7%) of 60 serum specimens from dogs (Table 1). Antibody titers ranged from 1:40 to 1:320. Positive sera were collected during January through October from dogs living in five coastal and inland towns in Connecticut (Darien, Deep River, East Hampton, Madison, and Middletown) and from two towns in New York State (Mt. Kisco and Wingdale). On the basis of veterinary records, the ages of the four male and three female seropositive dogs ranged from 1 to 8 years. Five of these dogs were lame, and of these, four had fever (rectal temperature, ≥39.5°C). The other two seropositive dogs had no signs of lameness or fever. The remaining 20 dogs with fever had no antibodies to *E. canis*. Three seropositive dogs with normal temperatures, however, had low erythrocyte counts (3.93×10^6 to 5.17×10^6 erythrocytes per μl^3). Total leukocyte counts for all seven seropositive dogs were normal. Information on platelet numbers for this group was unavailable. Six dogs lacking antibodies to *E. canis* had limb or joint disorders and anemia, with total erythrocyte counts ranging from 3.93×10^6 to 5.2×10^6 erythrocytes per μl^3. One other seronegative dog without signs of lameness had anemia. Leukopenia was noted for two additional seronegative dogs without lameness; total leukocyte counts of 5,100 and 5,300 leukocytes per μl^3 were recorded. Another dog, without serologic evidence of ehrlichiosis, had anemia (2.78×10^6 erythrocytes per μl^3) and leukopenia (5,900 leukocytes per μl^3). Blood cell counts for the remaining 43 dogs showing nonspecific signs of illness, 23 of which had limb or joint disorders, were normal.

TABLE 2. Reactivities of serum samples collected in 1985 from equids in Connecticut and tested by IFA staining methods for antibodies (total immunoglobulin) to *E. risticii*

Sampling period	Total no. of serum specimens	No. (%) positive	Geometric $\bar{x}$[a]	No. with antibody titers of:	
				80–320	640–1,280
April	19	0			
May	28	3 (10.7)	36.3	0	3
June	45	0			
July	54	9 (16.7)	35.9	6	3
October	17	4 (23.5)	40.3	4	0
November	24	1 (4.2)	26.2	1	0
Total	187	17 (9.1)	30.8	11	6

[a] A value of 25, the average titer for negative sera, was used for each negative sample in the analyses.

Serum specimens from equids contained antibodies to *E. risticii*. Of the 187 samples tested, 17 (9.1%) were positive (Table 2). Antibody titers usually ranged between 1:80 and 1:320, but maximal titers of 1:1,280 were recorded for specimens collected during May and July 1985. These animals lived in Cheshire (n = 2), Colchester, and Litchfield. The remaining equids with lower titers of antibodies to *E. risticii* lived in the following towns: Easton, Fairfield, Guilford, Berlin, Mansfield, Morris, Ridgefield, Scotland, Wallingford, Westbrook, and Westport (n = 3).

To assess the specificity and reproducibility of test results, groups of serum specimens were reanalyzed by IFA staining methods. Little or no cross-reactivity was noted. For example, seven dog serum specimens contained antibodies to *E. canis* in preliminary analyses. None of these reacted to *E. risticii* (Table 3) in the duplicated tests. When 10 equine serum specimens containing antibodies to *E. risticii* were screened against *E. canis*, 5 were seropositive. Antibody titers to *E. risticii* (1:160 to 1:1,280) were at least fourfold greater than those to *E. canis* (1:40 to 1:160). Similar results were obtained when positive controls were analyzed. Homologous titers to *E. canis* and *E. risticii* were at least eightfold greater than heterologous titers. In the reanalyses of seronegative dog (n = 28) and equine (n = 47) specimens, there was no reactivity to *E. canis* or *E. risticii*. The reproducibilities of the original assay results were also verified when the seropositive dog and equine specimens were retested against the respective homologous ehrlichial antigens. Antibody titers varied by twofold or less.

TABLE 3. Reactivities of dog and equine sera with or without antibodies to *E. canis* or *E. risticii* to *B. burgdorferi* by IFA staining methods

Study group[a]	Total no. of serum specimens tested	No. (%) positive for:		
		E. canis	*E. risticii*	*B. burgdorferi*[b]
Dogs, antibodies to *E. canis*	7	7 (100)	0	7 (100)
Dogs, no antibodies to *E. canis*	28	0	0	10 (35.7)
Equids, antibodies to *E. risticii*	10	5 (50)	10 (100)	0
Equids, no antibodies to *E. risticii*	47	0	0	10 (21.3)

[a] Study groups were established on the basis of preliminary analyses of sera. Samples were retested against antigens to determine specificity and reproducibility.

[b] Dogs and equids lived in tick-infested areas endemic for Lyme borreliosis; serum specimens were tested for total immunoglobulins. Some results were reported earlier (15, 17) and are listed here for comparison.

Results of IFA analyses from earlier studies (15–17) were reviewed to determine whether antibodies to *B. burgdorferi* had been detected in canine and equine sera. Each of the seven dog serum specimens reactive to *E. canis* by IFA staining methods also contained antibodies to *B. burgdorferi*. Titers to this spirochete ranged between 1:128 and 1:2,048 (16). Ten of 28 dog serum specimens seronegative for *E. canis* contained antibodies to *B. burgdorferi* in similar concentrations. However, the positive control serum specimen for *E. canis*, having a homologous titer of 1:8,192, had no antibodies to *B. burgdorferi*. In IFA staining analyses of 10 equine serum specimens with antibodies to *E. risticii*, none had reacted to *B. burgdorferi* (17). In similar tests of 47 serum specimens, which had no antibodies to *E. risticii*, 10 serum specimens had been found earlier to have antibodies to *B. burgdorferi*; antibody titers ranged from 1:128 to 1:2,048. The positive and negative control serum specimens for *E. risticii* were negative when tested for antibodies to *B. burgdorferi*.

DISCUSSION

Dogs and equids in Connecticut and the lower Hudson River Valley region of New York State had antibodies to ehrlichiae. Although the specific travel histories for many of these animals were unknown, infections were probably acquired in or near the towns where these animals lived. Canine ehrlichiosis was previously reported for a Brittany spaniel that lived in Milford, Conn., and had not traveled outside the state (19). Clinical signs for this dog included anemia, thrombocytopenia, lymphopenia, and splenomegaly. Serologic analyses detected antibodies to *E. canis* (titer, 1:2,560). In the same study, analyses of sera from 146 healthy dogs revealed no antibodies to *E. canis*. Moreover, canine ehrlichiosis is known to occur in Massachusetts and New York State (7). Our results for seropositive equids also confirm earlier findings (22, 25), which documented equine monocytic ehrlichiosis in Connecticut, New York State, and other northeastern states.

Clinical evidence of canine ehrlichiosis in the present study was inconclusive. Anemia in three dogs with antibodies to *E. canis* might have been due to *E. canis* infection. However, 10 additional dogs without antibodies to *E. canis* had pronounced anemia and/or leukopenia. Other dogs carrying antibodies to *E. canis* had no apparent signs of canine ehrlichiosis. Clinical presentation of this disease varies (9, 27), and without supportive laboratory data, diagnosis can be especially difficult. Depending on the breed of dog, the dog's immune status, and phase of infection, dogs may be acutely ill or lack signs of disease (subclinical) or they may develop a fatal syndrome characterized by high fever, thrombocytopenia, leukopenia, anemia, or hemorrhage. Immune responses also vary. In some dogs with *E. canis* infection, high concentrations of antibodies persist for several weeks or months, while in other dogs, antibody titers decrease sharply (26). When cases are suspected on the basis of clinical findings, laboratory analyses for hyperproteinemia and hypergammaglobulinemia should be included, along with determinations of antibody concentrations and erythrocyte, leukocyte, and platelet counts (27).

The clinical manifestations of equine monocytic ehrlichiosis can be mild or severe. Signs of disease can vary from fever, depression, and anorexia to severe diarrhea and laminitis (22). Like *E. canis* infections, conversion from seronegative to seropositive status for antibodies to *E. risticii* is not always accompanied by clinical signs of disease (25). Those investigators (25) showed that clinically undetected infections existed in experimentally inoculated horses.

The occurrence of canine and equine ehrlichiosis correlates with the presence of hard-bodied ticks. *R. sanguineus*, a known vector of *E. canis* (3), has widespread distributions on different continents. Although the arthropod vector of *E. risticii* is unknown, *I. scapularis*, *D. variabilis*, and *Amblyomma americanum* (Lone star tick) abound in areas where equine monocytic ehrlichiosis has been reported. The onset of cases of illness in equids during warmer months (20, 22) is further evidence for a tick or other arthropod vector. Experimentally, Lone star ticks transmitted *Ehrlichia ewingii*, a canine granulocytic *Ehrlichia* species, to dogs (2), but American dog ticks did not transmit this agent. Moreover, unidentified hemocytic rickettsia-like microorganisms with serologic reactivity to fluorescein-conjugated and unlabeled antisera to *E. canis* have been detected in *D. variabilis* and *I. scapularis* in Connecticut (18). These hemocytic rickettsia-like microorganisms coexisted with *B. burgdorferi* in 36 (6.7%) of 537 *I. scapularis* ticks examined. Isolation of *Ehrlichia* agents is required to determine whether *E. canis*, *Ehrlichia equi*, *E. ewingii*, *Ehrlichia platys*, *E. risticii*, or other ehrlichiae infect these or other ticks.

Although there is limited availability of some ehrlichial agents, such as *E. equi*, *E. ewingii*, and *E. platys*, multiple antigens should be included in serologic studies whenever possible. Simultaneous infections of *E. canis* and *E. platys* can occur (5). *E. platys*, which infects platelets in dogs and can cause cyclic thrombocytopenia, has widespread distribution in the United States (27). However, there is no convincing evidence that this agent is present in New England. In tick-infested sites, domesticated animals may become infected with multiple pathogenic organisms. The seven dogs with antibodies to *E. canis* and *B. burgdorferi*

were probably exposed to both agents, but these infections were not necessarily concomitant. Nonetheless, there is potential for simultaneous infections with ehrlichiae and *B. burgdorferi* in domesticated animals in New England. Moreover, on the basis of our results, there appears to be no cross-reactivity when antisera to *E. canis* and *E. risticii* (i.e., positive controls) were tested with *B. burgdorferi* antigen. This should help separate ehrlichiosis from Lyme borreliosis. The minor cross-reactivity with heterologous ehrlichial antigens noted in serologic analyses of *E. canis* and *E. risticii* antisera is consistent with previous findings (4, 25). In conclusion, serologic testing of canine and equine sera in conjunction with other hematologic analyses can help confirm clinical diagnoses and better define the geographic distribution of ehrlichiosis in these animals. In many tick-infested areas of Connecticut and New York State, ehrlichiosis coexists with Lyme borreliosis. Further surveillance for *E. canis* and *E. risticii* infections is warranted.

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