The Distribution of Canine Exposure to *Borrelia burgdorferi* in a Lyme-Disease Endemic Area

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ABSTRACT

Objectives. A serosurvey of canine exposure to *Borrelia burgdorferi*, the causative agent of human Lyme disease, was conducted in Westchester County, New York, to determine the distribution of exposure in an area endemic for Lyme disease.

Methods. A total of 1446 blood samples was collected from resident dogs and tested by modified enzyme-linked immunosorbent assay. Equivocal samples were further tested by immunoblot. A mean number of 57.8 samples was collected from each of 25 towns and cities.

Results. Seroprevalence rates for municipalities ranged from 6.5% to 85.2%. County seroprevalence was 49.2%. There was a significant difference among the rates for the northern (67.3%), central (45.2%), and southern (17.3%) regions. Multiple range analysis indicated homogeneity between the southern and central regions and the central and northern regions.

Conclusions. Canine exposure to *B burgdorferi* increases in a south to north gradient within the county. Intensity of exposure, measured by enzyme-linked immunosorbent assay titers, indicates a similar pattern. The close association between dogs and humans suggests that human risk of acquiring Lyme disease within Westchester County is equally disparate and is inversely related to the degree of urbanization. (*Am J Public Health.* 1993;83:1305–1310)

Introduction

Lyme disease is an infectious disease caused by a spirochete, *Borrelia burgdorferi*,[1,2] and transmitted by ticks of the *Ixodes ricinus* complex.[3] It has been reported in 46 states in the United States[4] and elsewhere throughout the world.[5–7] Lyme disease is particularly prevalent in the northeastern United States,[4,8] where the deer tick, *Ixodes dammini*, is abundant.[9–11]

Lyme disease can be difficult to diagnose,[12] and delayed treatment may lead to serious sequelae.[13–15] Prevention of infection, through public education or control of ticks,[16–18] is therefore an important objective. However, prior to the initiation of any prevention strategy, areas presenting human risk must be accurately identified.

Because of the expansion of Lyme disease into new areas,[4,8] as well as the apparent focal nature of tick populations in endemic areas,[11] it is often difficult for public health officials to monitor the risk and spread of the disease. To our knowledge, the most common method currently used by health agencies to monitor this risk is the tabulation of human cases reported by physicians.[4,8] However, relying on sporadic, nonstandardized case reporting by physicians may provide a biased interpretation of Lyme disease risk.

Unlike other tick-borne diseases of public health importance (i.e., Rocky Mountain spotted fever), exposure to Lyme disease in the Northeast is primarily of a peridomestic nature.[19,20] This suggests that methods of assessing risk should be particularly sensitive in residential areas.

Lyme disease in dogs has been reported since 1984.[21] It is known that dogs are frequently exposed to tick bites[22,23] and develop antibodies to *B burgdorferi*.[22,24,25] It has therefore been postulated that dogs, through serosurveys for *B burgdorferi* antibodies, may be useful in predicting human risk for Lyme disease.[26–28] However, no published reports of canine serosurveys are currently being used in local systematic studies of the geographic distribution of exposure within areas endemic for Lyme disease.

Westchester County (area = 1166 km^2), located in southern New York State, is an area where Lyme disease is endemic.[29] *I dammini* is the most common host-seeking tick in both residential[19] and recreational areas[11] and is the most prevalent tick parasitizing humans in the county.[20] A previous serological study conducted on dogs living in undefined areas of Westchester County and adjacent Connecticut reported an enzyme-linked immunosorbent assay seroprevalence rate of 89.6% in apparently healthy dogs (n = 48).[25] The purpose of our study was to measure the prevalence of canine exposure to *B burgdorferi* and to determine the geographic distribution of exposure

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This paper was accepted February 22, 1993.

within the county. Because of the close association between dogs and humans, these results will assist in defining the distribution of human Lyme disease risk on a countywide level.

Materials and Methods

Regional Breakdown

The county was divided into three areas—north, central, and south—to allow a comparison of serologic results on a regional level. These divisions were determined by grouping towns and cities based on their relative location so that regional sample sizes approached equality (north = 9, central = 9, south = 7), with the criterion that more than half of a particular town or city lies in its designated region.

Selection of Dogs

Canine blood samples were collected from August 16, 1989, through June 28, 1990. Samples were taken from dogs treated by 25 participating veterinarians distributed evenly throughout the northern, central, and southern regions of the county to ensure adequate coverage of all municipalities. Veterinarians were instructed to draw blood from dogs on a biweekly basis. Sampling involved taking aliquots of blood from all dogs being bled for any purpose (i.e., heartworm testing) on the first 2 days of the collection week. Dogs were not solicited specifically for this study.

Blood was collected in 4-cm^3 vacuum tubes containing separator gel and immediately refrigerated. Veterinarians were provided with data forms to record background information on each dog, including owner's name, town of residence, and travel history.

Processing of Samples

Samples were collected from veterinarians on alternating weeks, with those in the central and south constituting one collection group and those in the north the other. Whole blood samples were centrifuged. Serum from each sample was transferred to a sterile tube, given a coded accession number, and shipped overnight from Valhalla, NY, to Albany, NY, for testing. All serologic testing was conducted blind with respect to the geographic origin of the samples.

Control Sera

Blood samples from areas considered nonendemic for Lyme disease, such as New York City, upstate New York (Albany and Rensselaer counties), Colorado, and Wyoming, were collected by local veterinarians to serve as controls. Samples were processed and tested blind in a manner identical to the Westchester County samples.

Antigens

B burgdorferi ATCC No. 35210 was used to determine the presence of antibodies to the Lyme disease organism. For specificity studies, we used the following antigens: *Treponema phagedenis* biotype Reiter (obtained from Dr. R. M. Smibert, Virginia Polytechnic Institute, Blacksburg, Va); *Leptospira interrogans* serovar canicola, Hond Utrecht IV strain; and serovar icterohaemorrhagiae, RGA strain. Both serovars, solubilized in Laemmeli's buffer at a concentration of 1 mg/mL, were received from Dr. G. Baranton, Institut Pasteur, Paris, France. *B burgdorferi* was grown in BSK II[30] for 5 to 7 days and processed for enzyme-linked immunosorbent assay and immunoblot assays as described.[31] *T phagedenis* was grown in PYG semisolid/Smibert's salt solution/serum and cocarboxylase medium for 5 to 7 days (Dr. R. M. Smibert, written communication, November 1988). The *T phagedenis* was then processed as described for *B burgdorferi*.

Serologic Analyses

A modified enzyme-linked immunosorbent assay method was used to test sera for the presence of *B burgdorferi* antibodies. Samples that were equivocal according to that test were also subjected to immunoblot, a more specific diagnostic test.[32] A preliminary study was performed to determine the minimum significant level of reactivity of the enzyme-linked immunosorbent assay test. This was done by comparing the two test results. One hundred serum samples were grouped according to enzyme-linked immunosorbent assay titers and then tested by immunoblot. An immunoblot test result was considered positive if three or more antigen bands were scored visually on probing with the conjugate. This comparison showed that sera with enzyme-linked immunosorbent assay titers of 800 or more were positive by immunoblot; the sera with titers of 200 and 400 were positive by immunoblot at a rate of approximately 50% and 75%, respectively. Therefore, serum was considered positive for antibodies to *B burgdorferi* if it had an enzyme-linked immunosorbent assay titer of 800 or more. All sera with titers of 200 and 400 were also considered positive if confirmed by immunoblot.

Enzyme-linked immunosorbent assay. Lyophilized antigens (*B burgdorferi* or *T phagedenis* [biotype Reiter]) were suspended (0.08-mg dry weight per mL) in 0.06 M carbonate buffer (pH 9.6), and the suspensions were sonicated for 10 seconds. Twenty-five microliters of antigen suspension (2 μg) was added to each well of a series of microtiter plates. The antigens were then force precipitated[31,33] with 100 μL of 0.05% sodium acetate in 95% ethanol at 4°C for 20 hours. The next day the wells were washed with 0.1 M phosphate buffered saline (pH 7.4) containing 0.03% tween 20 (phosphate buffered saline–tween) and then blocked with phosphate buffered saline containing 3% bovine albumin. Canine sera (first antibody) were diluted to 1:100 in phosphate buffered saline containing 3% albumin. The first antibody (50 μL) incubation with antigen was at 37°C for exactly 30 minutes. The reaction was probed with a second antibody, goat anti-dog immunoglobulin (IgG) conjugated with horseradish peroxidase, at 37°C for exactly 30 minutes. After each incubation, the wells were washed with phosphate buffered saline–tween. The color reaction (o-phenylenediamine) was allowed to develop for 30 minutes and then read in a microtiter reader. Sera that scored an optical density of 0.3 or more after subtracting the average optical density obtained from three determinations of a nonreactive control were titrated. The antibody titer was the highest dilution that had an optical density between 0.2 and 0.3 after subtracting the nonreactive control at that dilution. The optical density of the nonreactive control at dilution of 1:200 or more was less than 0.1. A number of the sera were also tested with *T phagedenis* (biotype Reiter) for specificity and with two leptospira serovars. *T phagedenis* is a spirochete commonly found in nature that has closely related epitopes with *B burgdorferi* and other spirochetes. Leptospiras are bacteria that can infect dogs.[34]

Immunoblot. Antigens were solubilized in solubilizing buffer at 37°C for 5 minutes, and the solubilized antigens were subjected to SDS-PAGE electrophoresis by means of the Laemmli buffer system.[35] Electrophoresis was carried out at 30 mA for 3 to 4 hours. Electroblotting on nitrocellulose membrane (NCP) was performed with 25 mM phosphate buffer at pH 7.4. Methanol was not used in the transfer buffer. The antigen bands transfer was done at 250 mA for 18 hours at 4°C.

The nitrocellulose membranes were then blocked with 10% nonfat dry milk in phosphate buffered saline at 37°C for 1 hour. The membrane was then put in the Miniblotter system (Integrated Separation Systems, Hyde Park, Mass) for multiple serum testing, and the canine sera diluted in phosphate buffered saline–milk were applied to the channel and incubated at 37°C for 30 minutes. The lanes were washed in phosphate buffered saline–tween and reblocked for 5 minutes, and the reaction was probed with the same conjugate as for the enzyme-linked immunosorbent assay. Color development was done with 4-chloro-1-naphthol.[36] The gradation of the color intensity of the bands was scored visually from 1+ (weak or shadow) to 4+ (strong). To be scored as positive, a band had to be 2+ or more in intensity.

Statistical Analysis

Seroprevalence rates were calculated for each town and city. These rates were determined by the combined results of enzyme-linked immunosorbent assay and immunoblot, sample size was used as the denominator. Regional seroprevalence rates were compared by analysis of variance on the normally distributed data (Kolmogorov-Smirnov goodness of fit test, $P > .05$). Tukey's multiple range test was performed to test for homogeneity of groups.[37] These comparisons were based on individual municipality seroprevalence rates excluding those municipalities where less than 20 samples were collected.

In comparing the intensity of exposure to *B burgdorferi* among dogs from each region, the distribution of reciprocal enzyme-linked immunosorbent assay titers in all three regions was examined by an $R \times C$ G-test of independence.[38] As a means of determining the significance of differences in exposure of dogs between regions, comparisons of each regional combination (i.e., north and central, north and south, central and south) were also made with the G-test.

Results

A total of 1607 blood samples was collected from Westchester County veterinarians, and 1446 were used in the study. The remaining 161 samples contained an insufficient amount of serum for testing, were damaged in shipping, or were collected from dogs not residing in the county. A mean of 57.8 (SE = 8.03) samples was obtained from the 25 municipalities, with the sample sizes ranging from 8 to 188. Mean sample sizes obtained from municipalities within the three regions were 66.7 (SE = 18.68) for the north (n = 600), 63.9 (SE = 9.15) for the central (n = 575), and 38.7 (SE = 9.67) for the south (n = 271).

TABLE 1—Seroprevalence of Canine Exposure to *Borrelia burgdorferi* in Towns and Cities of Westchester County, New York, 1989 through 1990

Town/City	Region	No. Samples	Positive, %
North Salem	North	27	85.2
Bedford	North	188	76.1
Mt. Kisco	North	102	70.6
Lewisboro	North	53	67.9
Somers	North	54	64.8
Yorktown	North	104	59.6
Cortlandt	North	8	50.0
Pound Ridge	North	33	51.5
Peekskill	North	31	38.7
North Castle	Central	94	78.7
New Castle	Central	94	68.1
Mt. Pleasant	Central	55	56.4
Ossining	Central	78	48.7
Greenburgh	Central	83	28.9
White Plains	Central	62	27.4
Scarsdale	Central	64	14.1
Harrison	Central	14	7.1
Rye (town)	Central	31	6.5
Rye (city)	South	45	28.9
Eastchester	South	12	25.0
Pelham	South	20	20.0
Mt. Vernon	South	12	16.7
Yonkers	South	46	15.2
New Rochelle	South	55	14.6
Mamaroneck	South	81	12.4
Total		1446	49.2

Control Sera

A total of 217 blood samples were collected from dogs residing in areas considered nonendemic for Lyme disease. All sera were tested by enzyme-linked immunosorbent assay and 43 were further examined by immunoblot. Nine (4.1%) were considered positive for *B burgdorferi* antibodies. Four of these positive dogs had a history of travel to areas in New York State and Pennsylvania endemic for Lyme disease. The travel history of the remaining five dogs is unknown.

Serologic Analyses

A total of 1446 samples was tested by enzyme-linked immunosorbent assay; 373 of these samples were subsequently tested by immunoblot. Individual municipality seroprevalence rates varied from 6.5% to 85.2% (Table 1). The overall county seroprevalence was 49.2%. Only six positive samples were collected from dogs with travel histories to areas outside of Westchester County endemic for Lyme disease. Results of the testing for cross reactivity with *T phagedenis* were essentially negative (less than 3 bands or bands that scored 1+ or less reaction) at all enzyme-linked immunosorbent assay titers examined. Similar results were obtained with the two Leptospira serovars.

TABLE 2—Comparison of Enzyme-Linked Immunosorbent Assay Titers with Positivity of the Immunoblot Test

Titer	No.	Positive by Immunoblot, %
≥1600	94	100
800	31	90
400	27	78
200	59	58
100	9[a]	0

[a]All nine sera had from one to three bands. However, no bands scored more than 1+. These sera were scored negative by immunoblot.

The results of the relationship between enzyme-linked immunosorbent assay and immunoblots are summarized in Table 2. For this study, enzyme-linked immunosorbent assay titers of 800 or more were considered positive; sera with titers of 200 to 400 were confirmed by immunoblot. The immunoblot was the determinant factor for these sera. Sera with enzyme-linked immunosorbent assay titers of 100 or less were considered negative because the bands, if present, were scored as 1+ or less reaction. As expected, the number of bands increased with the high titers. The most common were at a relative mobility of approximately 98, 80, 68, 54, and 42 kd.

Regional Comparisons

Regional seroprevalence rates were calculated as 67.3% in the north, 45.2% in the central region, and 17.3% in the south. Analysis of variance on the seroprevalence rates of towns and cities with 20 or more samples resulted in a significant difference ($F = 9.484$, $P < .01$) among the northern (n = 8), central (n = 8), and southern (n = 5) regions. Tukey's multiple range analysis indicated the presence of two homogeneous groups (south/central and central/north). These results suggest that canine exposure to *B burgdorferi* is not uniform within the county and increases in a south to north gradient (Figure 1).

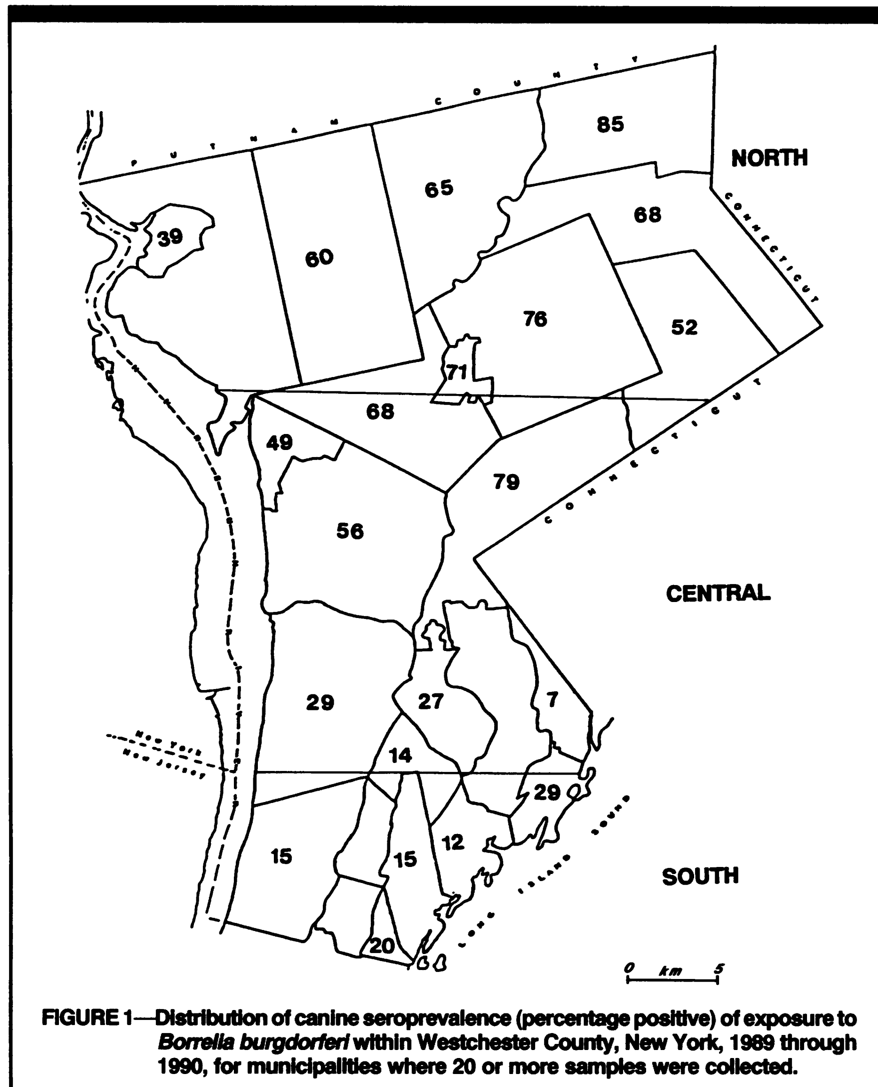


FIGURE 1—Distribution of canine seroprevalence (percentage positive) of exposure to *Borrelia burgdorferi* within Westchester County, New York, 1989 through 1990, for municipalities where 20 or more samples were collected.

Distribution of Titers

The distribution of the magnitude of enzyme-linked immunosorbent assay titers was calculated for the three county regions, with the north region having a greater proportion of higher titers (Figure 2). Results of the R × C G-test of independence comparing all regions were significant ($P < .05$), indicating that the frequency of titers is dependent on region. The distributions of titers in all pairwise comparisons (i.e., north and central, north and south, central and south) were also significantly different ($P < .05$).

Discussion

Previous efforts to study canine exposure to *B burgdorferi* by serologic testing have taken place primarily over large geographic areas: New Jersey,[26] North Carolina,[27] Texas,[39] Wisconsin,[40] Massachusetts,[41] western France,[42] and Hokkaido, Japan.[43] Although these studies have provided information regarding regional exposure of dogs to *B burgdorferi*, they have not involved sufficient sample sizes within a relatively small geographic area to make accurate conclusions about exposure on a local level.

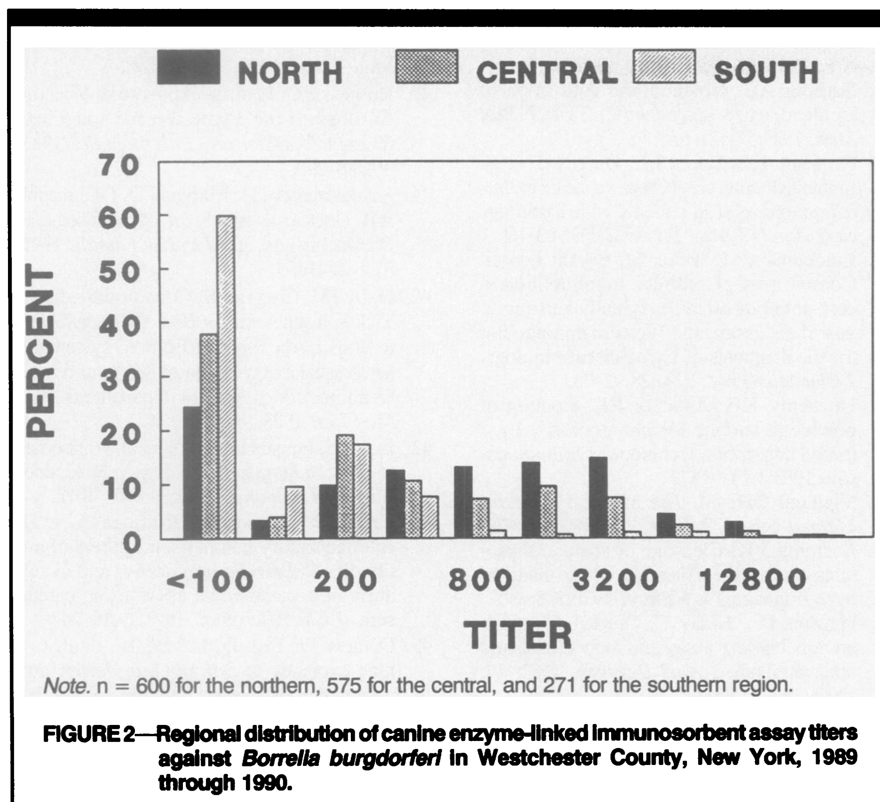
In the present study, 1446 canine blood samples were collected from dogs residing within Westchester County. The resulting map of canine exposure to *B burgdorferi* (Figure 1) provides a measure of exposure at the town and city level.

The results of this study indicate that canine exposure to *B burgdorferi* is not homogeneous within Westchester County; rather, it increases significantly from south to north. This is supported by regional differences in both seroprevalence (Figure 1) and intensity of exposure (Figure 2). These results suggest that there is a cline of increasing seropositivity from south to north. Factors that influence this distribution of exposure are probably related to the distribution and abundance of *I dammini* and are inversely related to the degree of urbanization. This is supported by the fact that the southern region of the county is more heavily populated (414 943 residents) than the more heavily wooded central (213 474 residents) and northern (246 449 residents) areas. Population density, a more reliable indicator of urbanization, shows an even greater disparity, with the southern region having 7544 residents per square mile. The central and northern regions have 1334 and 1049 residents per square mile, respectively.

Despite the increased level of exposure associated with the northern region, our results suggest that there is some canine exposure to *B burgdorferi* in all municipalities located within the county. Even in the southern region, seroprevalence rates of 6.5% for Rye, 12.4% for Mamaroneck, and 14.6% for New Rochelle are higher than the 3.3% reported from North Carolina[27] and the 5.5% from Texas,[39] where the numbers of reported Lyme disease cases are low.[8] The fact that only 9 of 217 (4.1%) control sera from nonendemic areas were positive, including 4 with a travel history to an endemic area, indicates that the serologic testing in our study was highly specific.

The overall county seroprevalence rate of 49.2% is consistent with the results reported in other studies conducted within areas endemic for Lyme disease. Burgess[40] reported a seroprevalence rate of 54.0% by indirect immunofluorescent-antibody assay in Wisconsin ($n = 380$). In the endemic area of Monmouth County, New Jersey, Schulze et al.[26] reported a rate of 42.6% by indirect immunofluorescent-antibody assay ($n = 202$). In the present study, the seroprevalence rate of 67.3% in the northern region of the county ($n = 600$) is appreciably higher than in other endemic areas. However, comparisons of results among serologic studies should be interpreted cautiously because of potential differences in serologic techniques[32] and variations among laboratories.[44]

It has been suggested that canine exposure to *B burgdorferi* may be an indicator of human risk for Lyme disease.[26–28] Our data demonstrating the nonuniform distribution of canine exposure therefore suggest that human risk may be equally disparate in Westchester County. This is supported by the distribution of reported human cases within the county. In the northern region, there were 214 confirmed cases per 100 000 population reported for 1990. In the central and southern regions, there were 81 and 18 cases per 100 000



Note. n = 600 for the northern, 575 for the central, and 271 for the southern region.

FIGURE 2—Regional distribution of canine enzyme-linked immunosorbent assay titers against *Borrelia burgdorferi* in Westchester County, New York, 1989 through 1990.

population, respectively. The possibility of a nonuniform distribution of risk is an important consideration when devising public health strategies to prevent and control Lyme disease. Knowledge of high-risk localities within an endemic area would greatly increase the efficiency of such programs.

Recent studies have attempted to examine the relationship between canine exposure to *B burgdorferi* and human risk for Lyme disease. Daniels et al.[45] reported a significant correlation between the distribution of canine seropositivity and human cases in endemic and nonendemic areas throughout the northeastern United States. Eng et al.[28] reported that in coastal Massachusetts, the prevalence of *B burgdorferi* infection was significantly greater in dogs (74% by indirect immunofluorescent-antibody assay; n = 34) than in people (approximately 30% by enzyme-linked immunosorbent assay; n = 64). The authors suggested that dogs are more sensitive indicators of the presence of ticks infected with *B burgdorferi*. Lindenmayer et al.,[41] in a study comparing canine seroprevalence and human Lyme disease in Massachusetts, concluded that estimates of the prevalence of canine exposure to *B burgdorferi* offer a reliable measure of the potential risk to people. Canine serology is an especially attractive Lyme disease surveillance method given (1) the problems associated with human case reporting, (2) that serological testing from other host animals can yield an unusually low seroprevalence,[46] and (3) that surveillance of vector ticks over a large area is extremely labor intensive. Although the relationship between canine exposure and human disease needs to be more clearly defined, we feel that the detection of canine exposure to *B burgdorferi* is currently the most sensitive method available to assess human risk for Lyme disease on a countywide level. □

Acknowledgments

This research was supported by research grant C-003852 from the Tick-Borne Disease Institute, New York State Department of Health, Albany.

Portions of this paper were presented at the Fourth International Conference on Lyme Borreliosis, Stockholm, Sweden, June 1990.

We thank T. Daniels for advice and comments, D. Aspros and E. Bosler for advice on study design, and the following individuals for providing blood samples: D. Aspros, A. Bergman, L. Berk, R. Bohning, J. Debbie, E. Feinberg, E. Fleischli, L. Fox, J. Freidman, A. Grossman, T. Hoch, M. Horowitz, J. Israel, R. Jakimer, J. Klinger, M. Leeds, A. Leitner, M. Marder, R. McLean, M. Meadow, L. Miller, M. Padilla, P. Raiti, M. Randell, A. Schreier, B. Strauss, E. Thorndike, M. Wolland, M. Woltz, D. Woolfe, G. Yarnell, and C. Zymet. We also thank A. Deguglielmo for providing human case information and S. Wear for providing area data used to calculate population densities.

References

1. Burgdorfer W, Barbour AG, Hayes SF, et al. Lyme disease—a tick-borne spirochetosis? *Science.* 1982;216:1317–1319.
2. Johnson RC, Schmid GP, Hyde FW, et al. *Borrelia burgdorferi* sp. nov.: etiologic agent of Lyme disease. *Int J Syst Bacteriol.* 1984;34:496–497.
3. Piesman J. Transmission of Lyme disease spirochetes (*Borrelia burgdorferi*). *Exp Appl Acarology.* 1989;7:71–80.
4. Centers for Disease Control. Lyme disease surveillance—United States, 1989–1990. *MMWR.* 1991;40:417–421.
5. Schmid GP. The global distribution of Lyme disease. *Rev Infect Dis.* 1985;7:41–50.
6. Chengxu A, Yuxin W, Yongguo Z, et al. Clinical manifestations and epidemiological characteristics of Lyme disease in Hailin county, Heilongjiang Province, China. *Ann NY Acad Sci.* 1988;539:302–313.
7. Kawabata M, Baba S, Iguchi K, et al. Lyme disease in Japan and its possible incriminated tick vector, *Ixodes persulcatus. J Infect Dis.* 1987;156:854.
8. Miller GL, Craven RB, Bailey RE, et al. The epidemiology of Lyme disease in the United States 1987–1988. *Lab Med.* 1990; 21:285–289.
9. Piesman J, Spielman A. Host-associations and seasonal abundance of immature *Ixodes dammini* in southeastern Massachusetts. *Ann Entomol Soc Am.* 1979;72:829–832.
10. Spielman A, Wilson ML, Levine JF, et al. Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. *Annu Rev Entomol.* 1985;30:439–460.
11. Falco RC, Fish D. Potential for exposure to tick bites in recreational parks in a Lyme disease endemic area. *Am J Public Health.* 1989;79:12–15.
12. Steere AC. Medical progress: Lyme disease. *N Engl J Med.* 1989;321:586–596.
13. Steere AC, Batsford WP, Weinberg M, et al. Lyme carditis: cardiac abnormalities of Lyme disease. *Ann Intern Med.* 1980;93:8–16.
14. Reik L, Steere AC, Bartenhagen NH, et al. Neurologic abnormalities of Lyme disease. *Medicine.* 1979;58:281–294.
15. Steere AC, Gibofsky A, Patarroyo ME, et al. Chronic Lyme arthritis: clinical and immunogenic differentiation from rheumatoid arthritis. *Ann Intern Med.* 1979;90:896–901.
16. Schulze TL, Parkin WE, Bosler EM. Vector tick populations and Lyme disease: a summary of control strategies. *Ann NY Acad Sci.* 1988;539:204–211.
17. Spielman A. Prospects for suppressing transmission of Lyme disease. *Ann NY Acad Sci.* 1988;539:212–220.
18. Stafford KC. Effectiveness of carbaryl applications for the control of *Ixodes dammini* (Acari: Ixodidae) nymphs in an endemic residential area. *J Med Entomol.* 1991;28:32–36.
19. Falco RC, Fish D. Prevalence of *Ixodes dammini* near the homes of Lyme disease patients in Westchester County, New York. *Am J Epidemiol.* 1988;127:826–830.
20. Falco RC, Fish D. Ticks parasitizing humans in a Lyme disease endemic area of

southern New York State. *Am J Epidemiol.* 1988;128:1146–1152.
21. Lissman B, Bosler EM, Camay H, et al. Spirochete-associated arthritis (Lyme disease) in a dog. *J Am Vet Med Assoc.* 1984; 185:219–220.
22. Magnarelli LA, Anderson JF, Kaufmann AF, et al. Borreliosis in dogs from southern Connecticut. *J Am Vet Med Assoc.* 1985; 186:955–959.
23. Daniels TJ, Fish D, Falco RC. Seasonal activity and survival of adult *Ixodes dammini* (Acari: Ixodidae) in southern New York state. *J Med Entomol.* 1989;26:610–614.
24. Magnarelli LA, Anderson JF, Schreier AB, et al. Clinical and serologic studies of canine borreliosis. *J Am Vet Med Assoc.* 1987;191:1089–1094.
25. Magnarelli LA, Anderson JF, Schreier AB. Persistence of antibodies to *Borrelia burgdorferi* in dogs of New York and Connecticut. *J Am Vet Med Assoc.* 1990;196:1064–1068.
26. Schulze TL, Bosler EM, Shisler JK, et al. Prevalence of canine Lyme disease from an endemic area as determined by serosurvey. *Zentralbl Bakteriol Mikrobiol Hyg (A).* 1986;263:427–434.
27. Greene RT, Levine JF, Breitschwerdt EB, et al. Antibodies to *Borrelia burgdorferi* in dogs in North Carolina. *Am J Vet Res.* 1988;49:473–476.
28. Eng TR, Wilson ML, Spielman A, et al. Greater risk of *Borrelia burgdorferi* infection in dogs than in people. *J Infect Dis.* 1988;158:1410–1411.
29. Williams CL, Curran AS, Lee AC, et al. Lyme disease: epidemiologic characteristics of an outbreak in Westchester County, NY. *Am J Public Health.* 1986;76:62–65.
30. Barbour AG. Isolation and cultivation of Lyme disease spirochetes. *Yale J Biol Med.* 1984;57:521–525.
31. Hechemy KE, Harris HL, Duerr MJ, et al. Immunoglobulin G subclasses specific to *Borrelia burgdorferi* in patients with Lyme disease. *Ann NY Acad Sci.* 1988;539:162–169.
32. Lindenmayer J, Weber M, Bryant J, et al. Comparison of indirect immunofluorescent-antibody assay, enzyme-linked immunosorbent assay, and Western immunoblot for the diagnosis of Lyme disease in dogs. *J Clin Microbiol.* 1990;28:92–96.
33. Hechemy KE, Anacker RL. Coating of polymeric surface for immunoassay by a forced adsorption technique. *J Immunoassay.* 1983;4:147–157.
34. Sigmund OH, ed. *The Merck Veterinary Manual.* 5th ed. Rahway, NJ: Merck; 1979.
35. Laemmli VK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* 1970;228:680.
36. Hawkes PC, Niday E, Gordon J. A dot-immunobinding assay for monoclonal and other antibodies. *Anal Biochem.* 1982;119: 1406.
37. Steele RGD, Torrie JH. *Principles and Procedures of Statistics.* New York, NY: McGraw-Hill; 1960.
38. Sokal PR, Rohlf FJ. *Biometry: The Principles and Practice of Statistics in Biological Research.* 2nd ed. San Francisco, Calif: Freeman; 1981.
39. Cohen ND, Carter CN, Thomas MA, et al. Clinical and epizootiologic characteristics of dogs seropositive for *Borrelia burgdorferi* in Texas: 110 cases (1988). *J Am Vet Med Assoc.* 1990;197:893–898.
40. Burgess EC. Natural exposure of Wisconsin dogs to the Lyme disease spirochete (*Borrelia burgdorferi*). *Lab Anim Sci.* 1986; 36:288–290.
41. Lindenmayer JM, Marshall D, Onderdonk AB. Dogs as sentinels for Lyme disease in Massachusetts. *Am J Public Health.* 1991; 81:1448–1455.
42. Doby JM, Chevrier S, Couatarmanac'h A. Tick spirochetosis by *Borrelia burgdorferi* in dogs in the west of France: systematic serological examination of 806 hounds and 88 military dogs from 14 departments. *Rec Med Vet.* 1988;164:367–374.
43. Isogai E, Isogai H, Sato N, et al. Antibodies to *Borrelia burgdorferi* in dogs in Hokkaido. *Microbiol Immunol.* 1990;34:1005–1012.
44. Greene RT, Hirsch DA, Rottman PL, et al. Interlaboratory comparison of titers of antibodies to *Borrelia burgdorferi* and evaluation of a commercial assay using canine sera. *J Clin Microbiol.* 1991;29:16–20.
45. Daniels TJ, Fish D, Levine JF, et al. Canine exposure to *Borrelia burgdorferi* and prevalence of *Ixodes dammini* (Acari: Ixodidae) on deer as a measure of Lyme disease risk in the northeastern United States. *J Med Entomol.* 1993;30:171–178.
46. Magnarelli LA, Anderson JF, Hyland KE, et al. Serologic analysis of *Peromyscus leucopus*, a rodent reservoir for *Borrelia burgdorferi*, in the northeastern United States. *J Clin Microbiol.* 1988;26:1138–1141.