CANINE SURVEILLANCE SYSTEM FOR LYME BORRELIOSIS IN WISCONSIN AND NORTHERN ILLINOIS: GEOGRAPHIC DISTRIBUTION AND RISK FACTOR ANALYSIS

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Abstract. A seroprevalence survey for Borrelia burgdorferi was conducted among the healthy canine pet population in selected counties of Wisconsin and northern Illinois to determine the distribution of Lyme disease and associated risk factors. Information obtained for each dog included place of residence, Lyme disease vaccination status, history of travel and tick exposure, signalment, and medical history. Serum samples were screened by enzyme-linked immunosorbent assay and confirmed by an immunoblot procedure. Seroprevalence by county ranged 0–40%, with the highest estimates from west-central Wisconsin. The spatial pattern was significantly correlated with human incidence of Lyme disease and with abundance of the tick vector, Ixodes scapularis. A geographic information system (GIS) was used to integrate environmental data with the location of the residences of the dogs to determine environmental risk factors. Seropositivity among dogs was positively associated with increased tick exposure and time spent outdoors and negatively associated with vaccination against Lyme disease. Seropositivity was also associated with living in forested and urban areas, and on sandy, fertile soils. A canine surveillance system is a useful method for assessing the geographic distribution of Lyme disease, and in combination with a GIS, it can be effective in determining environmental factors associated with I. scapularis endemcity.

INTRODUCTION

Lyme disease, the most common vector-borne disease in the human population in the United States, is caused by the spirochete Borrelia burgdorferi and is transmitted by the black-legged tick Ixodes scapularis in the northeastern and midwestern United States. Borrelia burgdorferi causes disease in humans and domestic animals, particularly dogs.2,3 Dogs are competent reservoirs for B. burgdorferi and once infected, they can produce antibodies that persist for at least 17 months.4

Various studies have assessed the distribution of Lyme disease on the basis of the prevalence of B. burgdorferi among symptomatic and asymptomatic dogs. Among 234 asymptomatic dogs from southern Connecticut, 53.4% had antibodies to B. burgdorferi.5 In a survey of symptomatic dogs in the northeast, 76.3% from southern New York and 66.5% from southern Connecticut were seropositive by ELISA or immunofluorescent assay (IFA).6 In New Jersey, 34.7% of asymptomatic dogs from 43 municipalities were seropositive.8 A serosurvey of 380 dogs from United States Department of Agriculture (USDA) vendors in Wisconsin revealed 53% seropositivity by IFA.3 In Michigan, 8% of 299 dogs from Menomonee County in the Upper Peninsula were seropositive.9 In these studies, ELISA and IFA were used for serological analysis, and in some studies, immunoblot analysis was applied to selected samples. Enzyme-linked immunosorbent assay and IFA are sensitive methods for screening; however, IFA is less sensitive and reliable than ELISA.10 The use of an immunoblot procedure to confirm positives may result in lower antibody seroprevalence estimates because of its higher specificity.11

Because dogs produce antibodies to B. burgdorferi (which are detectable up to 2 years4), have a travel history, and are exposed to the outdoor environment frequently, it has been postulated that they can be used as sentinels for an active surveillance program for Lyme disease.9,12,13 Canine seroprevalence estimates have been compared with results obtained through other methods of surveillance. In Massachussetts, a significant correlation was found between human incidence of Lyme disease and canine seroprevalence.13 Also, a survey of 2 coastal areas showed that the prevalence of B. burgdorferi infection was greater in dogs than in people.12 In Westchester County, New York, the geographic distribution of seroprevalence increased from south to north, related to a decrease in urbanization.14 The distribution of I. scapularis was associated with canine seropositivity, with greater numbers of I. scapularis ticks and seropositive dogs found near the coastline of Maine.15 In a study encompassing 5 northeastern states, the prevalence of black-legged ticks on white-tailed deer was positively correlated with canine B. burgdorferi seropositivity, and this association was used to map the risk of acquiring Lyme disease.16

In addition to describing the geographical distribution of Lyme disease, it is important to determine if there are any risk factors, both intrinsic and extrinsic, that can increase the risk of acquiring Lyme disease. Several studies have identified risk factors associated with canine seropositivity. A logistic regression model was used to determine that large, mixed-breed dogs, dogs older than 2 years, and dogs residing at altitudes < 70 m were more likely to be seropositive.10

A geographic information system (GIS) was used to identify environmental variables associated with an increased risk of Lyme disease for human patients in Maryland. Residence in forested areas and on well-drained, deep, loamy soils were significant risk factors.14 In Illinois, increased tick densities on white-tailed deer were found in forested areas with sandy soils near rivers,19 and in Wisconsin, a higher incidence of human cases was seen in counties with greater forest cover.20 In these states, populations of I. scapularis reservoir hosts, such as white-tailed deer and white-footed mice, are ubiquitous in wooded areas.21 Therefore, host densities do not appear to be a limiting environmental factor for the tick population.22
In this study, we used a canine survey to determine *B. burgdorferi* antibody seroprevalence and a GIS to study the spatial distribution of Lyme disease risk and its environmental determinants at the county and address level in Wisconsin and northern Illinois. Our objective was to test the use of dogs as sentinels in an active surveillance program for Lyme disease.

**MATERIALS AND METHODS**

**Sample population.** Counties in Wisconsin and northern Illinois (Bestudik J, personal communication) were selected according to a previous history of Lyme disease or the presence of *I. scapularis*. Adjacent counties were then added to determine the borders of distribution. All veterinarians in the counties listed in the American Veterinary Medical Association directory were contacted by mail and asked to participate in the study. Each participating clinic was requested to submit 20 serum samples from healthy, asymptomatic dogs; most samples were obtained from dogs screened for heartworm disease. The only restriction placed on sampling was for half the dogs to weigh < 15 kg and half to weigh more. On the basis of an average of seroprevalence rates reported in the Northeast, a sample size of 20 dogs per county was determined by power analysis to detect a prevalence of 30 ± 20% (P < 0.05). Serum samples were obtained from clinics in 25 counties in Wisconsin and 7 counties in northern Illinois. Veterinarians and pet owners were asked to complete a questionnaire for each dog.

**Questionnaire.** Information was obtained for age, weight, sex, and breed of each dog. Breeds were divided into a mixed-breed group and the 7 groups recognized by the American Kennel Club (sporting, herding, working, terrier, toy, nonsporting, and hound) to reduce the number of categories. For function, dogs were classified as pets or as hunting, herding, or guard dogs. For habitat, the categories were 100% indoors, 100% outdoors, and 50%/50%. Dogs were considered to have traveled if they had been outside their county of residence in the previous 3 years. Medical history was obtained for antibiotic use and the presence of any symptoms (fever, joint pain, or swelling) consistent with Lyme disease. Owners and veterinarians were asked if any ticks (regardless of species) had ever been found on the dog, whether any tick control products were being used, and if the canine Lyme vaccine had been administered. To determine the possibility of cross-reactions with leptospires and oral treponemes, the status of vaccination against leptospirosis and the presence of dental tartar were ascertained.

**Enzyme-linked immunosorbent assay.** An ELISA similar to that described by Magnarelli and others and Lindenmayer and others was used to detect serum antibodies to *B. burgdorferi*. Sera were diluted 1:320 to reduce cross-reactivity with heterologous antibodies. The antigen used in the ELISA was a heat-killed, sonicated *B. burgdorferi* spirochete suspension (Kirkegaard and Perry, Gaithersburg, MD) diluted 1:8,000 in blocking buffer. The detecting substrate was TMB-ELISA (tetramethyl benzidine; Life Technologies, Gaithersburg, MD).

Reactions were stopped after incubation for 30 min with 2 N HSO₄, and optical density of wells was read at 450 nm with an ELISA plate reader (Molecular Devices, CA). Samples were considered positive if optical density values were ≥ 3 standard deviations above the mean optical density of negative controls. The end-point titers of positive sera were determined by 2-fold serial dilutions. Positive control sera with a high titer (> 1:1,280) were from a site in northern Michigan or were obtained courtesy of Dr. L. Magnarelli of the Connecticut Agricultural Experiment Station (New Haven, CT).

Negative control canine sera were from dogs that met the following criteria: no history of vaccination for Lyme disease, no history of tick bite, no suspect diagnosis of Lyme disease, no periodontal disease, previous negative test by IFA for antibodies to *B. burgdorferi* (< 1:160), and not residents of a known endemic area. We used 6 positive and 6 negative control sera on each microtiter plate, and all sera were tested in duplicate.

**Western blot.** Western blot analyses were performed on all samples shown to be positive by ELISA with the Lyme Immunostrip IgG Assay kit (Immunetics, Cambridge, MA). Aliquots (10 μL) of undiluted canine serum samples were blotted onto test strips in channels containing 1 mL of dilution buffer. Antigens on membranes of this kit were separated by the manufacturer and include the following 18 bands: p93, p66, p58, p45, p41, p39, p37, p35, p34, p31, p30, p28, p23, p18, p17, p15, and p14. Visualization of specific protein bands indicated the presence of serum IgG against *B. burgdorferi* antigens. Samples were classified as positive or negative according to the criteria established by Immunetics. Positive samples were then analyzed by a logistic regression model to distinguish between vaccinated and naturally infected dogs. The bands generally indicative of natural exposure by this analysis are p58, p37, p35, and p30. The bands generally indicative of vaccination, and not natural exposure, are p93, p34, p31, and p28.

**Geospatial analysis.** The residential locations of dogs were entered into a GIS (ARC/INFO, ESRI, Redlands, CA) to create a georeferenced database (Figure 1). The resulting map was overlaid with geographic coverages of land cover–land use and elevation obtained from WISCLAND (University of Wisconsin/Wisconsin Department of Natural Resources, Madison, WI) and Illinois GIS (Illinois Department of Natural Resources, Springfield, IL). Soil data, including order, texture and drainage, were obtained from STATSGO (USDA, Washington, DC), with a resolution of 2.5 km². The land cover–land use coverage was divided into 4 categories: forest, urban, grassland, and agriculture. Elevation, which ranged 156–462 m, was divided into 5 equal categories. Soil order showed of the 8 of the 12 soil orders described for Wisconsin and northern Illinois. Mollisols (present under prairie) and alfisols (deciduous forests) are moist, fertile soils with distinct layers and < 25% organic matter. Spodosols (coniferous forests) also have distinct layers, but they are acidic, with decreased fertility. Less fertile soils include the alluvial entisols and the clay-enriched vertisols. Inceptisols are also less fertile, but they are moist and acidic. Histosols
containing > 25% organic matter are found in bogs and swamps. Soil texture was divided into 7 groups according to particle size, ranging from clay (< 2 μm) and silt (2–50 μm) to sand (0.05–2 mm).33 Soil drainage capacity was divided into 6 categories, from very poorly drained to well drained.33

**Descriptive analysis.** Canine seroprevalence for antibodies to *B. burgdorferi* was calculated according to county. At the county level, the Pearson correlation was used to compare seroprevalence with the proportion of dogs vaccinated. Means and standard deviations were calculated for continuous variables, including age and weight. The proportion of dogs in each category of the categorical and dichotomous variables was determined. Associations among tick exposure, vaccination, and use of tick control products by land cover category were analyzed by Spearman rank order correlation. At the county level for Wisconsin, canine seroprevalence was calculated for each of the 61 counties, with sample sizes ranging 5–73 dogs per county. Prevalence by county ranged 0–40%, with the highest prevalence detected among counties in west-central Wisconsin (Figure 2). In Illinois, seropositive dogs were found in Winnebago, JoDaviess, and LaSalle Counties. Canine seroprevalence by county in the present study was correlated posi-

**Canine risk factor analysis.** Univariate analyses were initially performed on all variables obtained from the questionnaire and the GIS coverages to determine any significant associations with seropositivity. Continuous variables (age and weight) were analyzed by Student’s *t*-test. Ordinal variables (elevation, soil texture, drainage, and soil order) were analyzed by Spearman rank order correlation. Chi-square analysis was performed on all dichotomous (sex, Lyme and leptospirosis vaccination status, tick exposure, tick control, antibiotic use, presence of dental tartar, and symptoms consistent with Lyme disease) and categorical variables (breed, function, habitat, and land cover–land use).

Variables with *P* < 0.2 were entered into a logistic regression model to determine which risk factors were significantly associated with seropositivity. Only variables with *P* < 0.05 were included in the final model. The outcome variable was positive (1) or negative (0) serological status. Dogs with a travel history outside of the county of residence were excluded from the analysis. The final model was derived by a backward stepwise approach. Logistic regression analysis was also performed excluding all vaccinated dogs to eliminate vaccination status as a possible confounder. All statistical analyses were performed by SPSS software (SPSS, Chicago, IL).

**RESULTS**

The response rate of the initial mailing to all veterinarians was 73%. The positive response rate was 46%. A total of 25 clinics in Wisconsin agreed to participate in the study, with dogs from 49 counties represented. In Illinois, 7 clinics agreed to participate, with dogs from 12 counties represented. The mean age of the dogs in the sample was 5.3 ± 3.4 years (range, 1–16 years). Their mean weight was 17.4 ± 11.3 kg (range, 2.0–61.8 kg). The sex ratio was 47.8% males and 52.2% females. The sporting group represented the greatest proportion of the breeds (28.3%), followed by the mixed-breed group (26.4%), with the remaining 5 groups ranging 5.4–10.2%. The majority of dogs were classified functionally by their owners as pets (88.0%), with 10.7% of dogs used for hunting and 1.3% used for herding or guarding. Most (59.7%) were classified as 100% indoor dogs, with 26.4% as 100% outdoor dogs and 14.0% as both. Most of the dogs resided in urban areas (35%), followed by residence in agricultural (30%), forested (19%), and grassland (16%) areas.

Screening by ELISA showed that 562 (52.2%) of 1,077 samples were positive. Immunoblot analyses of 562 positive samples yielded 101 samples classified as negative and 461 samples with one or more bands indicative of natural exposure, vaccination, or both. By use of an algorithm developed by Guerra and others,11 105 of the ELISA-positive samples were classified as naturally infected, and 372 were classified as vaccinated. The 16 samples classified as both were considered seropositive when entered into the logistic regression model. A total of 1,010 samples were used in the analysis after excluding dogs with a history of travel.

Canine seroprevalence was calculated for each of the 61 counties, with sample sizes ranging 5–73 dogs per county. Prevalence by county ranged 0–40%, with the highest prevalence detected among counties in west-central Wisconsin (Figure 2). In Illinois, seropositive dogs were found in Winnebago, JoDaviess, and LaSalle Counties. Canine seroprevalence by county in the present study was correlated posi-
with the abundance of *I. scapularis*/H11005 from expected values, indicating a good fit of the final model. The goodness-of-fit test showed no significant deviation from the final model are shown in Table 2. The Hosmer-Lemeshow test showed a good fit of the final model.

The highest percentage of dogs exposed to any ticks, as reported by owners, was in forested areas; these dogs also had the highest percentages of vaccination and use of tick control products (Table 1). The lowest percentage of tick exposure was in urban areas. When dogs were categorized according to the land cover of their place of residence, the proportion of dogs exposed to ticks was significantly correlated with vaccination and use of tick control products (r = 0.93 and r = 0.98, respectively; P < 0.001). However, when evaluated at the county level, the percentage of seropositivity was not significantly correlated with percent vaccination (r = 0.13, P = 0.2). Owners may therefore be vaccinating their dogs and using tick control products as a result of finding ticks, not because they are residing in an *I. scapularis*-endemic area.

All independent variables (P < 0.2) were entered into the logistic regression model except sex, breed, and habitat (time spent outdoors). The significant risk factors (P < 0.05) of the final model are shown in Table 2. The Hosmer-Lemeshow test showed no significant deviation from expected values, indicating a good fit of the final model.

### DISCUSSION

The risk of Lyme disease to dogs and humans in a region is directly related to the presence of infected ticks. However, the environmental determinants of this risk are not well known. Because dogs spend more time outdoors than people and have greater contact with ticks, they are a better indicator of exposure to infected ticks and are suitable sentinels for assessing the risk of Lyme disease regionally. Here, the

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**Figure 2.** Canine *Borrelia burgdorferi* seroprevalence (%) in selected counties of Wisconsin and northern Illinois.
distributed in the study counties of Wisconsin was congruent with the known distribution of vector ticks and human incidence of disease in the same region.20 Thus, our results support the hypothesis that dogs can be used as part of a regional surveillance system for Lyme disease to determine risk of disease to humans as well as dogs.

Seropositive dogs were found in southeastern counties east of the Wisconsin River (Figure 1), indicating that the distribution of Lyme disease is continuing to spread eastward, as reported previously.24,25 In addition, seropositive dogs were found in LaSalle County, Illinois, which has previously been considered not at risk for Lyme disease.35 The finding of seropositive dogs in counties where Lyme disease has been considered to be of low or no risk for humans20,24,25,35 indicates that serological analysis is a sensitive method for surveillance. The results of the present study suggest that surveys for I. scapularis could be undertaken in counties considered to be low-risk to no risk to confirm its presence and abundance.

The logistic regression model determined that several risk factors for dogs were significant (Table 2). Dogs that were outdoors for long periods of time hunting or herding were more likely to be seropositive for antibodies to B. burgdorferi. Pet dogs, which have a more limited home range, were less likely to be positive, regardless of amount of time spent outdoors. An association between seropositivity and reported tick exposure was found, with greater tick exposure occurring in forested and urban areas. These areas contain trees, shrubs, and leaf litter, creating a more suitable habitat for I. scapularis ticks than grasslands and land used for agriculture. Application of tick control products on dogs did not appear to be effective in controlling tick infestation and subsequent B. burgdorferi infection.

When individual dogs were considered, use of the Lyme vaccine was negatively associated with seropositivity. However, at the county level, the percentage of dogs vaccinated was not associated with seroprevalence estimates. Use of the vaccine varied by clinic, not by county; thus, the administration of Lyme disease vaccine was not related to actual risk of disease. Logistic regression analysis, which allows for the determination of individual risk, indicated a protective effect due to vaccination. The county level correlation analysis did not, illustrating that inferences at the population level cannot necessarily be applied to the individual level.

Comparisons among the land cover categories indicated that the percentages of tick exposure, Lyme vaccination, and use of tick control were lowest in urban areas, intermediate in agricultural zones and grasslands, and highest in forested areas (Table 1). Tick exposure encompassed reports of any species of tick; therefore, reports of ticks on dogs residing in grasslands and agricultural areas could be attributed to the presence of other species, such as Dermacentor variabilis and Rhipicephalus sanguineus. The increased seroprevalence in forested areas, in spite of a greater proportion of vaccinated dogs and increased use of tick control products, may be explained by dogs already being seropositive before vaccination and the application of tick control products. It could also be attributed to a recent increase in preventive measures in response to a greater awareness of the risk of contracting Lyme disease, which may occur if cases are newly diagnosed in an area.

In this study, the use of a GIS allowed for the integration of georeferenced locational and environmental data to determine associations between environmental factors and B. burgdorferi seropositivity. The results of this study in the Midwest support the findings of other studies from the Northeast14,15 and suggest which factors may be necessary for tick survival in the environment. A significant association for seropositivity was found with increasing elevation. In Wisconsin and Illinois, higher elevations are found in areas that were once covered by glaciers, and associations between glacial outwash and tick endemicity have been suggested.30 However, because the distribution is still expanding, this association may be transient as the tick population expands to the south and east from the northwestern focus. Seropositivity was associated with living in forested and urban areas, which includes suburban, residential communities. Previous studies have determined that I. scapularis has a preference for wooded habitats. In a survey of residential sites in Connecticut, nymphs and larvae were mostly present in woodland plots, whereas adults were collected from areas bordering the forest and from lawns.31 In Westchester County, New York, larvae were also found mostly in woods, but nymphs and adults were found in all habitats, including ecotone, ornamental vegetation, and lawns.38 The same pattern was observed on Fire Island, New York, during the fall season.39

Seropositivity was also associated with the fertile soils that underlie deciduous forests and prairie grasslands. These soils, which contain greater amounts of calcareous material and organic matter, are more suited for supporting biological activity.32 Ixodes scapularis may survive better in soils containing higher amounts of organic matter that is associated with increased amounts of leaf litter.40 Removal of the leaf litter layer resulted in significantly decreased numbers of nymphs and larvae,41 so it appears to be an important component of the microclimate. Soil particle size was another significant risk factor and is related to the water-retaining properties of the soil.31 Sandy soils have better drainage and have been associated with tick endemicity in Illinois.33 In Wisconsin, soils of medium to fine particle size are usually saturated at the time of spring thaw when adults begin questing, and tick survival may be adversely affected in soils with greater proportions of silt and clay. This study suggests that a combination of interrelated environmental factors is involved in determining the optimal habitat for I. scapularis.

The results of this study support the use of dogs as part of a surveillance system for Lyme disease. The geographical distribution of canine seroprevalence was similar to human incidence and tick endemicity, which are usually determined through passive surveillance by state health departments. Canine surveillance has the potential of being an active system because most pet dogs give blood samples yearly, their residences and travel history are known, and they have increased exposure to their local environment. Temporal changes in the distribution of B. burgdorferi seropositivity can be monitored by repeated sampling of current study participants, and changes in the spatial distribution can be ascertained by sampling dogs from currently nonendemic areas.
When the residential locations of the subjects are known, environmental factors associated with canine seropositivity can be identified. Seropositivity in a dog that has not traveled indicates that its residential area is a suitable habitat for *I. scapularis* populations and reservoir hosts, and that the ticks and hosts are infected with *B. burgdorferi*. Use of a GIS allows for the association of environmental factors with specific locations. Environmental and epidemiological data can then be combined to create risk profiles for the acquisition of Lyme disease or other tick-borne diseases, and identifying environmental factors that increase the risk for Lyme disease transmission can facilitate the detection of unknown foci and the prediction of where new foci may emerge. Knowledge of the geographic distribution of Lyme disease and associated environmental risk factors obtained through a canine surveillance system can facilitate the development of prevention and control programs for the human population.

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