Dogs as Sentinels for Lyme Disease in Massachusetts

BSTRACT

Background. An investigation of the relationship between incident human cases of Lyme disease and seroprevalence of antibodies to B. burgdorferi in dogs was undertaken in order to determine whether dogs might serve as sentinels for Lyme disease.

Methods. 3011 canine serum samples were analyzed by ELISA for antibody to B. burgdorferi. Records of incident human cases of Lyme disease were obtained from the Massachusetts Department of Public Health.

Results. Regression analyses of the relationship between the log_{10} (mean incidence in people 1985-1989) and canine seroprevalence from July 1988-August 1989 revealed that canine seroprevalence was highly predictive of incidence (R^2 = 0.86 , $p < 0.001$). A logistic regression model that incorporates the altitude of the town where each dog was resident, the date of sampling, and information on each dog's age, sex, and breed adequately explained the risk of canine seropositivity. Dogs resident at altitudes less than 200 feet, of sporting or large mixed breeds, and greater than two years of age were five times, four times, and almost three times more likely, respectively, to exhibit seropositivity than were other dogs.

Conclusions. Estimates of the prevalence of antibody to B. burgdorferi in dog populations offers a sensitive, reliable, and convenient measure of the potential risk to people of B . burgdorferi in the environment. Risk factors for canine seropositivity may directly or indirectly illuminate certain aspects of the epidemiology of human Lyme disease. (Am J Public Health. 1991;81:1448-1455)

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Introduction

Summary statistics of incident human cases collected by the Centers for Disease Control and state departments of public health have been used to highlight national and regional distributions and changes in Lyme disease epidemiology. 1-3 Passive surveillance systems, however, are well known for underestimating actual cases of any disease. The implementation of measures designed to minimize human exposure to ticks and a geographically variable index of suspicion for Lyme disease may affect numbers of case reports, but they have little to do with changes in the environmental burden of B. burgdorferi.

Testing dog populations for the presence of antibody to B . burgdorferi has been used as an epidemiologic tool to supplement surveillance based on passive case reporting.4-10 Dogs in endemic areas have been shown to be almost six times as likely as people to be exposed to infected ticks,1' making them a sensitive indicator of the presence of B . burgdorferi. The present study was undertaken to determine whether a relationship exists between seroprevalence of antibodies to B. burgdorferi in the dog population and incident cases of human Lyme disease in Massachusetts. If so, dogs might serve as sentinel animals for Lyme disease.

Methods

Canine sera

Thirty-two veterinary practices located in eight Massachusetts counties participated in this study (Figure 1). Practices were chosen on the basis of the following criteria: routine use of Tufts Veterinary Diagnostic Laboratory (TVDL), willingness to participate in the study for the period of

¹ year, and location. Practices were chosen to represent counties that were known, based on Massachusetts Department of Public Health records, to be endemic for Lyme disease (Dukes, Nantucket, Essex, Barnstable, and Plymouth counties), to represent areas where Lyme disease activity has recently been reported (Hampshire and Hampden counties), or to be located in areas where Lyme disease had not previously been reported (Worcester).

At each veterinary practice, veterinarians were asked to choose a convenient day of the week, and on that day to take serum samples from the first 5 to 10 consecutive dogs that were seen, regardless of the reason for presentation. If a client did not permit blood to be drawn, the veterinarian skipped that dog and continued to sample consecutively until the predetermined number of samples had been collected. For each dog sampled, veterinarians filled out brief forms providing information on the dog's breed, age, sex, owner'^s name and address, reason for presentation to the veterinarian, date the sample was obtained, and whether or not the dog was ^a Lyme disease suspect.

Sampling was undertaken from August 1, 1988, through December 31, 1988,

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suspended for the winter, begun again on April 1, 1989, and continued through July 31, 1989. Samples were collected in serum separator tubes and sent to the TVDL where they were centrifuged and frozen at minus 20C until testing could be performed. Serum samples from dogs suspected of having Lyme disease were tested within 7 days of arrival, and serum samples from dogs not suspected of having Lyme disease were tested within 4 months of arrival at TVDL. The presence of antibodies to B . burgdorferi was measured using an enzyme-linked immunosorbent assay (ELISA) as previously described.12 The cutoff value for seropositivity of 0.170 optical density units is 3 standard deviations (SDs) above the mean optical density for serum samples taken from 34 apparently healthy dogs resident in areas not endemic for Lyme disease.

Human Lyme Disease Cases

Statistics on Lyme disease cases for the years 1985 to 1989 were obtained from the Surveillance Service of the Division of Epidemiology in the Massachusetts Department of Public Health.

Statistical Analysis

Canine seroprevalence was calculated as the number of dogs seropositive divided by the number of dogs tested for each category of interest. A logistic regression model was developed to estimate relative risks for canine exposure associated with five factors that were suspected, a priori, of being predictive of seropositivity.

1. Altitude of the town where each dog was resident. Several environmental factors have been proposed to explain the range of *Ixodes dammini*.¹³⁻¹⁶ The distribution of incident cases of human Lyme disease in Massachusetts suggests an association between altitude, either directly or as a proxy for other factors, and incidence. Altitude was modeled on two levels depending on whether a town was located at $<$ 200 feet or \geq 200 feet above sea level.

2. Date the sample was drawn. Nymphal ticks, the developmental stage most responsible for transmission of B. burgdorferi to people, have been demonstrated to be most active in late spring and early summer, whereas adult ticks have been most active in the fall and winter.17 The sampling date was thus modeled on two levels, depending on whether a sample was obtained between August ¹ and December 31, 1988, or between April ¹ and July 31, 1989. These dates roughly correspond to fall/winter and spring/ summer seasons.

3. Sex. This factor was modeled on two levels, male and female, regardless of whether a dog was intact or neutered/ spayed. Previous studies indicate that male dogs may be predisposed to exposure to *B. burgdorferi.*⁹

4. Breed. Three levels of breed were chosen to reflect the relative probability of TARI F 1-Comparison of Annual Incidence^a of Human Lyme Disease with Canine Seroprevalence,^b 1988-1989, by Massachusetts County

Note. Barn = Barnstable; Berk = Berkshire; Brist = Bristol; Frank = Franklin; Hshire = Hampshire; Mid = Middlesex; Nant = Nantucket; Ply = Plymouth; Worc = Worcester;- = no cases reported/no dogs tested.

^aCases/100 000 persons (Source: Massachusetts Department of Public Health).

^bNumber seropositive/number tested × 100.

"Mean incidence, 1985-1989.

^aCI = confidence interval.

^eRepresents a single case

exposure to ticks in the environmentsporting and large mixed breed dogs (high probability of exposure), toy and small mixed breed dogs (low probability of exposure), and all others, includingworking, hound, terrier, nonsporting, medium mixed breeds, and dogs of unknown breed status (intermediate probability of exposure).

5. Age of the dog at the time of sampling. The risk for dogs of testing positive for antibody to B . burgdorferi has been found to be associated with the cumulative time of *I. dammini* exposure,¹¹ although other studies have reported a greater degree of seropositivity and higher antibody titers among younger than among older dogs.6.9 Age of dogs, as a proxy for cumulative time of exposure, was modeled on two levels, ≤ 2 years of age and >2 years of age.

Logistic regression analyses were carried out with BMDP and SAS, and Pearson χ^2 analysis was performed with StatXact (Cytel Software Corporation, Cambridge, Mass).

Results

Characteristics of the Study Population

During the period August 1, 1988, to July 31, 1989, 3011 serum samples obtained from 2941 Massachusetts dogs

were tested for the presence of antibody to B. burgdorferi. Seventy dogs, or 2% of the total population, were sampled twice, the second time for reasons unrelated to the present study. Males and femaleswere approximately equally represented (46.4% vs 49.5%, respectively). Sex of the remaining 4.1% of the study population was not noted byveterinarians. Sporting, large mixed breed, andworking dogs comprised the majority of the breeds represented (29.4%, 24.3%, and 19.6%, respectively). All other breeds were approximately equally well represented. The percentage of dogs in each age category ranged between 5.7% and 8.9% up to 11 years of age. Above that, the percentage in each age category ranged between 0.1% (>15 years old) and 4.7%.

Seroprevalence

Among all dogs tested, unconditional seroprevalence was 20.3%.

Comparison of incidence and prevalence. Canine seroprevalence was calculated by dogs' place of residence, the most likely site of exposure to B . burgdorferi. This permitted inclusion of 262 of 351 Massachusetts communities in the analysis.

Great variation exists among incident human cases by county from year to year (Table 1). By contrast, canine seroprevalence datawere available for every county in the state except one, and, because so manydogs were sampled, reasonable confidence intervals could be calculated for each point prevalence estimate. A comparison of incidence and prevalence by town for a single county (Essex) likewise confirms the completeness and stability of the canine data (Table 2).

Regression analyses of the relationship between canine seroprevalence in 1988 to 1989 and human incidence per 100 000 persons for each year between 1985 and 1989 by county were unable to statistically confirm the utility of canine seroprevalence as a predictor of the number of human cases. This is likely attibutable to the small number of documented county-specific cases in anygiven year. In order to overcome the variability inherent in the human data, cases of Lyme disease were averaged by county for the years 1985 through 1989. When the relationship between log_{10} (mean incidence in humans 1985-1989) and canine seroprevalence was explored, canine seroprevalence was found to be highly predictive of incidence $(R^2 = 0.80, P < .0001)$ (Figure 2).

Seroprevalence by month. Seroprevalence by month of sample submission did not vary with the exception of November 1988, when a particularly high proportion of samples exhibited seropositivity (Figure 3).

TABLE 2-Comparison of Annual Incidence of Human Lyme disease^a with Canine Seroprevalence,^b 1988-1989, in towns of Essex **County, Massachusetts**

Town	Human Year					Dog	
						Year	
	85	86	87	88	89	88 89	(95% CI ^c)
Andover	3.7 ^d				3.7 ^d	0.029	$(-0.027 - 0.085)$
Beverty		2.6 ^d		5.3	2.6 ^d	0.057	$(-0.020 - 0.134)$
Boxford			17.8 ^d			0^e	
Danvers					œ	0.088	$(-0.007 - 0.183)$
Essex					65.8	0.677	$(0.563 - 0.791)$
Georgetown						0.167	$(-0.044 - 0.378)$
Gloucester						0.294	$(0.186 - 0.402)$
Groveland					18.9 ^d		
Hamilton					14.5 ^d	0.452	$(0.277 - 0.627)$
Haverhill						0 ^e	
Ipswich	35.4	123.9	44.2	26.5	35.4	0.350	$(0.202 - 0.498)$
Lawrence	—	1.6 ^d					
Lynn			1.3 ^d			0.077	$(-0.025 - 0.179)$
Lynnfield						0.083	$(-0.073 - 0.239)$
Magnolia						0.333	$(0.025 - 0.641)$
Manchester			18.6 ^d			0.100	$(-0.031 - 0.231)$
Marblehead						0 ^e	
						0 ^e	
Methuen						0.043	$(-0.040 - 0.126)$
Middleton							
N. Andover						0.091 0 ^e	$(-0.079 - 0.261)$
Nahant							
Newbury		21.1 ^d					
Newburyport		m		12.1		0.200	$(-0.151 - 0.551)$
Peabody					4.5	0.024	$(-0.023 - 0.071)$
Rockport					31.5	0.263	$(0.065 - 0.461)$
Rowley					—	0.333	$(-0.044 - 0.710)$
S. Hamilton					---	0.222	$(0.065 - 0.379)$
Salem		2.7 ^d	2.7 ^d			0.031	$(-0.029 - 0.091)$
Salisbury						0.200	$(-0.151 - 0.551)$
Saugus						0°	
Swampscott		7.3 ^d		14.6		0 ^e	
Topsfield						0.167	$(-0.005 - 0.339)$
W. Peabody						0 ^e	
Wenham		25.8 ^d	25.8 ^d	æ.	51.7	0.200	$(-0.151 - 0.551)$

Note. - = no cases reported/no dogs tested.

a Cases per 100 000 persons (Source: Massachusetts Department of Public Health).

^bNumber seropositive/number tested × 100.

°CI = confidence interval.

^dRepresents a single case

^eRepresents testing of ≥5 dogs.

Seroprevalence by breed. Seropositivity was highest among sporting and large mixed breed dogs and lowest among toy and small mixed breed dogs (Figure 4).

Seroprevalence by age. Degree of seropositivity was lowest among dogs ≤ 2 years of age. Above 2 years of age, seropositivity remained relatively stable (Figure 5).

Sensitivity of the Canine Model

Using seropositivity alone, far more dogs tested positive for exposure to B. burgdorferi than incident human cases were reported. Comparing mean incidence for 1985 to 1989 with canine seroprevalence by county, seropositivity estimates were 513 to 17 857 times greater than incident human cases.

Veterinary suspicion for Lyme disease was associated with serologic test results. Of 203 dogs that were noted to be Lyme disease suspects based on clinical presentation, 91 (44.8%) tested positive for exposure to B . burgdorferi, whereas only 18.4% (465/2530) of nonsuspects and 20.1% (56/278) of dogs whose Lyme disease status was not noted tested positive for exposure. The Pearson χ^2 test statistic was 141.7 (df = 2, $P = .0011$) and was based on a Monte Carlo simulation of 2000 tables generated from fixed marginal distributions of the observed data.

Logistic regression. Of the dogs studied, 2789 met the criteria that comprise the population profiles for the logistic regression model and were included in the analysis. The standard logistic regression model had the following form:

 $ln(p/1-p) = -4.74 + 1.64T - 0.20D$ $- 0.05S + 0.82B1 + 1.41B2 + 1.03A,$ where

 $p =$ probability of testing positive

 $1-p =$ probability of testing negative

- $T =$ altitude of town where dog was resident
- $D =$ season serum sample was obtained
- $S =$ sex of dog
- $B1 =$ indicator variable for the differential effect of moderate vs low exposure by breed
- $B2 =$ indicator variable for the differential effect of high vs low exposure by breed

Seroprevalence, for 13/14 Massachusetts Counties.

$A = age of dog at time of sampling$

The χ^2 goodness-of-fit statistic for the equation sum[2 O_i \times ln(O_i/E_i)] was 34.37 (df = 39, $P = .68$), suggesting that the model adequately explained the test outcome. The 48 possible cells resulting from the coding of the five factors yielded two cells without any observed data due to incomplete records. Two of the 46 remaining cells proved to be particularly unusual and not well accommodated by the logistic model. These two cells were low-exposure breed female dogs <2 years of age, resident at low altitudes and sampled in the spring or summer $(n = 3)$, and lowexposure breed male dogs \geq years of

age, resident at high altitudes and sampled in the fall or winter $(n = 5)$. These cells, because of their small counts, are unlikely to be representative of the corresponding population profiles. For this reason, they were removed from the analysis. The recalculated χ^2 goodness-of-fit statistic was 29.84 (df = 37, $P = .825$). All final results are based on the latter model.

Odds ratios for factors included in the model (Table 3) revealed that dogs resident at or near sea level were approximately five times as likely to test positive for exposure as dogs resident at higher elevations. Breeds with moderate probability of exposure and those with high

 \Box inficant. Sex of the dog had no effect on probability of exposure were more than two times and more than four times as likely, respectively, to test positive than breeds with low probability of exposure. Linear contrast analysis for differences between breeds with high probability of exposure and all others indicated that the former were far more likely to be exposed than the latter $(\chi^2 = 33.01, P = .0001)$. Dogs >2 years of age were almost three times as likely to test positive as dogs ≤ 2 years ofage. Dogs sampled in the springor summer were slightly less likely than dogs sampled in the fall or winter to test positive, although this difference was not sigthe probability of testing positive.

> In the logistic regression model, cells with the highest probabilities for seropositivity (>0.22) represented dogs that lived at altitudes <200 feet, of the sporting or large mixed breed types, and older than 2 years of age. No differences with respect to date of sampling or sex were noted. Cells with the lowest probabilities for seropositivity (<0.017) represented dogs that lived at altitudes ≥ 200 feet, were ≤ 2 years of age, and were of low and moderately exposed breed types. As before, no differences in date of sampling or in sex were noted.

Discussion

The dog has been proposed for use as a sentinel animal to detect the presence of B. burgdorferi for two reasons. Dogs exposed to infected ticks develop antibodies to the spirochete and often exhibit clinical signs of the disease similar to those in people. Second, dogs are more likely to be exposed to infected ticks because they come into more physical contact with tick habitats than do most people, and because measures to prevent exposure to ticks are difficult to implement in dogs. Results of the present study indicate that under certain conditions canine seroprevalence estimates are highly predictive of incident human cases, further strengthening the usefulness of dogs as sentinel animals.

The present comparison between two different measures of disease and two different time periods is unavoidable for two reasons. Incident canine cases of Lyme disease are difficult to determine. Efforts to induce seroconversion to B. burgdorferi or clinical Lyme disease in laboratory dogs have not been unifornly successful.18,19 Furthermore, dogs often exhibit seropositivity to the spirochete without concomitant clinical evidence of disease.^{6-8,11,18-21} It is unlikely, then, that incident cases among dogs will ever be available for comparison with human incidence rates. Second, regression analyses of canine seroprevalence in 1988-1989 and human incidence for each year between 1985 and 1989 failed to reveal a relationship between the two. This is probably due to the great variability inherent in incident human cases from year to year. Seroprevalence estimates among dogs for a given year are likely to be stable because so many dogs can be sampled under an active surveillance system. It would, however, be useful to extend these studies over a longer period of time in order to determine whether or not these observations are maintained.

The overall seroprevalence estimate of 20.3% for this study does not reflect seroprevalence among all Massachusetts dogs because participating veterinary clinics were chosen to afford maximal coverage of particular counties in Massachusetts. For this reason, county- and townspecific seroprevalence rates are more likely than state-wide estimates to be representative of dogs presented for veterinary care in these regions. However, all seroprevalence estimates may actually underestimate true seroprevalence because they were based on dogs presented toveterinary practices rather than the general canine population. Inclusion of stray dogs and dogs that do not receive regular veterinary care might well increase seroprevalence estimates because these dogs would be more likely to run free and therefore be exposed to infected ticks.

Seroprevalence estimates were based on the assumption that seropositive dogs received exposure in the towns where they resided. In fact, dogs often travel with their owners, particularly in the summer months. This raises the pos-

sibility that individual seropositive dogs were exposed to infected ticks at locations other than their towns of residence and might account for the presence of seropositive dogs in towns where Lyme disease is not known to be endemic.

Previous studies in this laboratory and elsewhere^{12,22-24} have documented false positive ELISA test results for both canine and human serum samples. While we recognize that this possibility exists, test results from an area where Lyme disease is not known to be endemic by Massachusetts Department of Public Health standards (i.e., Worcester County) indicate that a very small proportion of dogs (1/78 or 1.3%) tested positive for antibody to B . burgdorferi, presumptive evidence that the proportion of false positive samples in this study is low.

Use of the canine to detect and quantify Lyme disease risk offers several advantages over the use of human incidence rates alone. Dog populations are particularly convenient to test for exposure to B. burgdorferi. Dogs are routinely brought to veterinarians in the spring for heartworm testing. Blood drawn for this purpose can likewise be made available for Lyme disease testing, obviating the need for a separate visit to the veterinarian. Furthermore, dogs tested at this time of year are

usually well animals presented for routine checkups and are thus more likely than dogs presented at other times of the year to be representative of veterinary clinic catchment populations.

Second, seroprevalence estimates in dogs not only reflect incidence rates in people, but they also appear to be a far more sensitive indicator of the presence of B. burgdorferi than do the latter. Seropositivity for every 100 000 dogs tested was consistently much higher than Lyme disease incidence per 100 000 persons.

Third, seroprevalence estimates in dogs are more likely to reflect the actual environmental risk of Lyme disease than are reports of incident human cases. Factors such as increasing awareness of Lyme disease may falsely increase incidence rates, whereas the use of measures to prevent exposure to ticks may actually decrease incidence rates apart from any changes in the environmental burden of B . burgdorferi. Measures designed to prevent dogs from exposure to potentially infected ticks are frequently unrewarding. Dogs' propensity to pick up large numbers of ticks and the difficulties associated with finding tiny I . dammini make tick exposure a near-certainty in endemic areas except, perhaps, for dogs that rarely venture outdoors.

Fourth, canine seroprevalence estimates permit a fairly complete representation of areas of potential risk to be drawn. As evidenced by the Massachusetts Department of Public Health data, collected under a passive reporting system, incidence measures for a particular county or town are frequently based on a single case report. Furthermore, marked year-to-year variability in incidence rates is frequently observed. Under an active canine surveillance system such as the one used here, additional information was made available to permit the calculation of point prevalence estimates and reasonable confidence intervals for 31 of 34 towns in one particular county (Essex) and for 13 of 14 counties.

The logistic model employed in the present study appears to adequately explain the risk of seropositivity among Massachusetts dogs and has defined certain risk factors for canine exposure, namely, altitude of town of residence, breed of dog, and age. Dogs that live at altitudes <200 feet above sea level appear to be at greater risk for exposure to B. burgdorferi. The fact that seroprevalence varies widely even at this altitude raises the possibility that ecologic factors such as humidity or temperature may affect deer tick survival and thus the risk of exposure to Lyme disease. Lyme disease is also known to occur in noncoastal areas of the United States where altitude may not be definable as in the present model.

Previous studies have not been able to confirm a breed disposition among seropositive dogs, although mixed breed dogs and hunting breeds were more frequently seropositive.9 The present study, perhaps because of a larger sample size, establishes that seropositive dogs are more likely to be sporting and large mixed breed dogs. These animals are presumably at greater risk of exposure because the former are used for hunting and sporting purposes and the latter, by virtue of their size, may be allowed more freedom to roam.

Dogs >2 years of age appear to be at greater risk of exposure than younger dogs. Other studies have documented maintenance of antibody titers in dogs months to years after initial exposure to the spirochete.20,21 Under these circumstances, seroprevalence could be expected to increase with advancing age. In the present study, however, age-specific seroprevalence estimates appear relatively stable above 2 years of age. It may be that, for a given dog population, only a fixed proportion of its members are at risk for exposure to B . burgdorferi. For example, a smaller proportion of dogs might be at risk in areas with large urban populations and strict leash laws than in rural areas with less restrictive leash laws. Dogs at risk may be exposed to infected I. dammini and seroconvert at an early age, and thus, after the critical age of 2 years, no increase in age-specific seropositivity would be noted.

In the present study, sex appeared not to explain seropositivity. Information on reproductive status of an animal, whether intact or neutered/spayed, was requested but frequently not provided. It is possible that reproductive status, as an indicator for proclivity to roam, might well be a risk factor for seropositivity, although this could not be determined in the present study.

The risk factors identified for dogs may directly or indirectly illuminate certain aspects of the epidemiology of human Lyme disease. Altitude can certainly be a risk factor for people. Age, as a proxy for cumulative duration of exposure, and breed, as a proxy for occupational or recreational exposure, are other factors that should be considered in models investigating the risk of Lyme disease in people.

A vaccine against B. burgdorferi has recently been made available for use in dogs (Borrelia burgdorferi Bacterin, Fort Dodge Laboratories, Fort Dodge, Iowa). Widespread use of such a vaccine could well compromise the ability of canine populations to serve as sentinel animals for Lyme disease. Vaccinated dogs would presumably be protected against field exposure to the spirochete and antibody titers in vaccinated dogs would be indistinguishable from those of naturally exposed dogs. The degree towhich the dog-owning public avails itself or the vaccine remains to be seen. However, the vaccine is likely to be put to greatest use in Lyme diseaseendemic areas where a sentinel system would be of least benefit. Dogs in nonendemic areas might well still serve as an early warning system for detection of the spirochete if they remain largely unvaccinated. \Box

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The Food and Nutrition Section of the American Public Health Association announces the availability of the Helen R. Stacey and Joseph A. Walsh awards for graduate education at the master's degree level in public health nutrition. Three awards are given consisting of \$2000 each. These awards must be used for the academic year 1992 to 1993. Applications must be submitted no later than May 1, 1992.

Nominations are being solicited for the Mary C. Egan

Award for Outstanding Professional Contributions and Service in Public Health Nutrition. This award carries with it a \$1000 honorarium. Nominations must be submitted no later than May 1, 1992.

Applications and nominations forms may be secured from Elvira Jarka, MS, RD, Chair, Awards Committee, F&NS, APHA, Lake County Health Department, 2303 Dodge, Waukegan, IL 60085.