Canine Lyme Disease in the United States: Correlation with Human Cases and Risk Factors for Dogs

by

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Abstract

Lyme disease is one of the most common vector-borne diseases in the United States. It produces signs and symptoms, and can cause serious and debilitating cardiac, neurologic and joint sequelae. Reported human cases have increased steadily since the disease was first identified in 1977. In 2002, nearly 24,000 cases were reported in the U.S. However, due to the nonspecific clinical signs and symptoms associated with this disease, and the passive surveillance methods used, Lyme disease is likely under-recognized and under-reported. Dogs are also susceptible to Lyme disease and show a similar clinical course as humans. Canine Lyme disease is also likely under-diagnosed due to the non-specific clinical signs and the costs associated with accurate diagnosis.

We conducted a study to determine the incidence of Lyme disease in dogs in the U.S. and if the geographical distribution of incident canine cases was predictive of human cases. In addition, we examined the risk factors for contracting Lyme disease among dogs, including age, sex, breed, elevation of the city of residence and Lyme disease vaccination status. We examined human incidence data reported to the Centers for Disease Control and Prevention (CDC) during 2002 and compared this to canine incidence from Banfield, The Pet Hospital® veterinary hospitals located in 37 states. We used linear regression analysis to determine if the canine incidence of Lyme disease among dogs seen at Banfield veterinary hospitals during 2002 was predictive of human incidence in these counties.

We found that, in our canine study population, dogs had twice the incidence of Lyme disease diagnosis as the human population in the U.S. during

2002. Canine incidence of Lyme disease was shown to be predictive of human disease. In addition, we found that sporting, herding breeds and other medium to large dogs in the non-sporting group were at higher risk for Lyme disease than small toy breeds, probably because of time spent outdoors and increased tick exposure. We also found that as dogs age, they are more likely to contract Lyme disease.

These findings suggest that dogs may prove to be good indicators for the presence of *Borrelia burgdorferi* and the tick vectors in an area, and of expansion of tick populations to areas where they have not previously been identified. This could be particularly helpful in targeting public education about the disease and tick avoidance in these non-endemic areas. In addition, these findings can help veterinarians target education efforts on tick avoidance.

Introduction

The disease and its impact

Lyme disease is one of the most commonly reported vector-borne diseases in the United States.[1] In 2002, nearly 24,000 human cases were reported to the Centers for Disease Control and Prevention (CDC). Lyme disease was first described in the United States as "Lyme arthritis" in 1977 due to a cluster of arthritis cases of unknown cause near Lyme, Connecticut.[2] Also called "Lyme arthritis" or "Lyme borreliosis" it is caused by the spirochete *Borrelia burgdorferi.* In humans it is a multistage, multisystem disease that can cause chronic, debilitating sequelae. Cost of medical treatment and lost productivity due to the disease are high. One study estimated a national expenditure in the United States of $2.5 billion (1996 dollars) over 5 years for both direct medical and indirect costs due to Lyme disease.[3]

Transmission of *B. burgdorferi*

Lyme disease occurs in North America, Europe and northern Asia, which is the range of *Ixodes ricinus* complex ticks, which serve as vectors.[4] The reservoirs of infection for *B. burgdorferi* are small rodents, especially the white-footed mouse (*Peromyscus leucopus*) and the chipmunk (*Tamias striatus*) in the northeast and north central regions and the dusky footed wood rat (*Neotoma fuscipes*) in the pacific coastal region of the United States.[4] Known competent vectors of *B. burgdorferi* in the United States are the blacklegged tick (*Ixodes*

scapularis) in the eastern and north central United States, and the western blacklegged tick (*Ixodes pacificus*) in the far western United States.[5]

I. scapularis has a three-stage life cycle that can take up to two years to complete, depending upon the geographic region in which they are located. In northern populations, the cycle takes two years, with *I. scapularis* adult females laying eggs in the early spring. Eggs hatch into larvae and begin to feed on small mammals, birds and reptiles in the late summer and early fall. They molt into nymphs and are dormant until the following spring. Nymphs feed on small mammals, birds and reptiles in the late spring and summer and molt into adults in the fall. Adults feed on large and medium sized mammals such as deer and domestic animals during mating in the fall and egg production in the early spring.[6] Southern populations can have generation times as short as one year, with larvae and nymphs feeding from January through September.

The transmission cycle in the western U.S. is more complicated. *I. spinipalpis*, the tick vector that maintains *B. burgdorferi* infection in the rodent population, does not feed on humans or domestic animals. *I. pacificus* larval and nymphal stages occasionally feed on rodents infected via this cycle and can, in turn, become infected. After molting to nymphs and adults, infected ticks can transmit the agent to humans. Larval and nymphal stages of *I. pacificus* prefer to feed on lizards, especially the western fence lizard (*Sceloperus occidentalis*). Lizards contain an agent in their blood that purges *B. burgdorferi* infections from feeding ticks. This is one explanation for the lower percentage of infected adult *I.*

pacificus ticks in the west (1 to 6%) compared with *I. scapularis* in the east with greater than 50% infection.[7]

Humans are incidental hosts and can be parasitized by all life stages of these ticks. However, humans are most likely to contract *B. burgdorferi* when bitten by nymphal ticks in the late spring and early summer in the eastern United States. In the far western region of the United States adult ticks feed throughout the year and may be more important in transmission of *B. burgdorferi* to humans than in the eastern United States.[4]

White-tailed deer (*Odecoileus virginianus*) are considered to play a major role as hosts for adult *I. scapularis*.[6] The increase in the white-tailed deer population in the eastern United States in recent years may explain the expansion of established populations of *I. scapularis* in the northeastern and north central regions of the country.[5] Exclusion of white-tailed deer results in a significant reduction in *I. scapularis* populations, but does not completely eliminate the tick, as other medium and large sized mammals also serve as hosts for the adult.[6]

B. burgdorferi infection in humans

The incubation period for *B. burgdorferi* infection can be from several days to a month from exposure to an infected tick until onset of clinical signs. Lyme disease in humans can be described in terms of early localized, early disseminated and late-stage manifestations. Early, localized manifestations can

include erythema migrans (a large, red, circular lesion with central clearing), mild constitutional symptoms and regional lymphadenopathy.[8]

Early disseminated manifestations include secondary skin lesions, severe malaise and fatigue, migratory pain in joints, tendons, bursae, muscle and bone. Splenomegaly and generalized lymphadenopathy may be seen. Early neurologic signs can include meningitis, cranial neuritis, Bell's palsy, motor or sensory radiculoneuritis and subtle encephalitis. Early cardiac signs include atrioventricular nodal block, myopericarditis and pancarditis. Conjunctivitis may also occur.[8]

Late-stage manifestations include acrodermatitis chronica atrophicans, localized scleroderma-like lesions, fatigue and keratitis. Late-stage musculoskeletal manifestations include prolonged arthritis attacks, chronic arthritis, peripheral enthesopathy, periostitis and joint subluxations below lesions of acrodermatitis. Late neurologic signs that may be seen include chronic encephalomyelitis, spastic parapareses, ataxic gait, subtle mental disorders and chronic axonal polyradiculopathy.[8]

A diagnosis of Lyme disease in humans is often based on clinical signs, especially in endemic areas. However, erythema migrans is absent in 20% to 40% of infected children and about 20% of adults.[9] Serological testing with either an enzyme-linked immunosorbent assay (ELISA) or indirect fluorescent antibody (IFA) test is recommended by the CDC. If equivocal or positive results are obtained, the more specific Western immunoblot (WB) test is conducted. If ELISA or IFA results are negative, no further testing is required. Patients in early

disseminated or late-stage disease will usually be strongly seropositive. But antibodies to *B. burgdorferi* can persist for months to years, so active disease cannot be determined by serology without the presence of clinical signs.[10] Definitive diagnosis of Lyme disease can be made by isolation of *B. burgdorferi* from erythema migrans skin lesion biopsy, blood or cerebrospinal fluid, but culture of the organism is difficult and requires special media.[11]

Patients treated in early stage Lyme disease, with appropriate antibiotics, usually recover rapidly and completely. Most patients treated in later stages also respond well to antibiotic treatment. Varying degrees of permanent damage to joints or the nervous system can develop in patients with late chronic disease. Antibiotics of choice for the treatment of early localized or early-disseminated Lyme disease, without neurologic involvement or third-degree atrioventricular heart block, are doxycycline or amoxicillin for 14 to 21 days. Parenteral antibiotics such as ceftriaxone are used in cases of neurologic disease and third-degree atrioventricular heart block.[12]

Prevention is accomplished by avoiding exposure to infected ticks by wearing long-sleeved shirts, long pants tucked into boots and using a DEET (n,n-diethyl-m-toluamide) containing insect repellant when in areas likely to be infested with ticks. In December 1998, the U.S. Food and Drug Administration licensed a human vaccine for Lyme disease, LYMErix®. The vaccine was derived from a lipidated outer surface protein (Osp A) of *B. burgdorferi.* In February 2002, the manufacturer removed the vaccine from the market, citing

poor sales, amid reports of some individuals developing severe arthritis after vaccination.

B. burgdorferi infection in dogs

Like humans, dogs have diverse clinical signs and symptoms, making diagnosis difficult in areas of the country where the disease is not considered endemic. The predominant clinical signs of *B. burgdorferi* infection in dogs are lameness and fever. Anorexia, fatigue and lymphadenopathy are also common. Lameness is caused by arthritis, which is often episodic, but can become chronic. Neurologic, cardiac, renal and reproductive signs have also been observed.[13]

Isolation of *B. burgdorferi* in blood, synovial fluid or urine is diagnostic, but difficult and therefore of little clinical value. Diagnosis is accomplished by exclusion of other causes of the clinical signs and serology. Serological techniques available are the IFA, ELISA and WB tests.[13] Canine Lyme disease vaccine complicates the interpretation of serologic results. Historically, the commercially available ELISA test was unable to distinguish between antibodies from natural infection and those produced to the vaccine.[14,15] In addition, other spirochetes, such as *Leptospira* species, can cause false positive reactions.[16]

The WB test has been used to differentiate between natural infection and vaccine through differences in immunoblot banding patterns. Differences in Western Blot protocols have produced interlaboratory variation in test results. These differences have been addressed to increase the specificity and sensitivity of human Lyme disease serodiagnosis, but there are no universally accepted criteria for dogs.[17] In March 2001, a new C_6 ELISA test, the SNAP-3 Dx Test®,

was introduced by IDEXX Laboratories. The SNAP-3 is an in-house test that incorporates tests for *B. burgdorferi*, *Ehrlichia canis* and *Dirofilaria immitis*. The test can distinguish between natural exposure and vaccinal antibody, and has been shown to be highly sensitive and specific for *B. burgdorferi* infection.[18]

Treatment of canine Lyme disease is similar to that in humans. Tetracyclines, amoxicillin or ampicillin at standard doses for 21 to 28 days have been used successfully.

As in humans, prevention is accomplished by avoiding wooded areas where ticks live, and by good tick control. Amitraz containing collars are effective, as are topical products such as fipronil and permethrins.[18] A killed whole cell *B. burgdorferi* vaccine became commercially available in 1990.[19] Since then several subunit vaccines have also been introduced. The use of vaccine is controversial. Some believe that vaccination is unnecessary, because most (95%) of dogs do not become ill after natural exposure to *B. burgdorferi*. Adverse reactions to the vaccine are considered moderate, with anaphylaxis sometimes reported. There is also concern about possible long-term immune-mediated sequelae, such as arthritis and neuropathy, associated with outer surface antigens such as OspA.[18]

Current surveillance system for Lyme disease in humans

The CDC initiated systematic surveillance for human Lyme disease in 1982. State health departments report cases of Lyme disease to the CDC through the National Electronic Telecommunications System for Surveillance

(NETSS). A standardized case definition was established in 1990 and implemented nationwide in 1991. A case of Lyme disease is defined as an illness consisting of either a physician diagnosed erythema migrans ≥ 5 centimeters in diameter, or at least one disseminated manifestation plus laboratory confirmation.[1] The laboratory testing protocol is cited above.

In 2002, 23,763 cases of human Lyme disease were reported to the CDC, an increase from 16,802 in 1998. Overall, more than 157,000 cases have been reported in the U.S. since 1982. The overall incidence rate of reported cases in the U.S. is approximately 7 per 100,000 population.[20]

The increase in reported cases is probably due to an increase in the true incidence of disease, as evidenced by the expansion of the range of both *I. scapularis* and *I. pacificus,*[5] as well as enhanced surveillance in the endemic areas of the northeastern and north central regions of the United States. However, true incidence may be higher due to under-reporting of the disease as shown in studies in Connecticut and Maryland, which have estimated 6 to 12 unreported cases for each reported case.[21,22] If these estimates are true, the reported human Lyme disease incidence in Connecticut during 2002 of 134 per 100,000 would be as high as 1204 per 100,000, and the Maryland incidence of 14 per 100,000 would be as high as 162 per 100,000. Under-reporting may be due to diagnosis often being based on clinical signs rather than laboratory findings. In non-endemic areas, lack of recognition of the often nonspecific clinical signs can also lead to underestimation of Lyme disease occurrence.

Studies on dogs as sentinels for human Lyme disease

The results of three studies indicate that seroprevalence of Lyme disease in dogs and human incidence in endemic areas are highly correlated.[23-25] This was also found in a study conducted in a non-endemic area (San Diego County, California).[26] However, larger, more diverse geographic areas have not been examined. In addition, seroprevalence for antibody to *B. burgdorferi* in canines has been examined in several states[24,26-34] and Canadian provinces.[35,36] Serological surveys have also been conducted in parts of Mexico,[37] Spain,[38,39] the Netherlands,[40] Japan[41] and Northern Bavaria.[42] However, the sampling and diagnostic methods varied among studies. In addition, no studies have been conducted that compare incidence of clinical Lyme disease in dogs and humans.

Risk factors for canine infection with *B. burgdorferi*

Risk factors for *B. burgdorferi* infection in dogs have been explored in several studies.[24-27,33,39-41] Age was evaluated as a risk factor in eight of these studies, with five finding that risk of being seropositive increased with increasing age. Nine studies evaluated breed or function of the dog as a risk factor.[24-27,31,33,39-41] Five of these studies showed that breed or use was a risk factor for exposure to Lyme disease. Sporting breeds,[24] hounds[41] and dogs used for hunting[25,31] and herding[25] had higher risk of exposure and developing antibody to *B. burgdorferi* than other breeds. Elevation of the dog's residence was examined in two studies with contradictory results. One study, conducted in 14 counties in Massachusetts, showed that dogs resident at elevations less than

200 feet above sea level were five times more likely to exhibit seropositivity than were other dogs.[24] The other, conducted in Wisconsin and Northern Illinois, showed that the odds of seropositivity increased with increasing elevation.[25] However, there has been no nation-wide study to examine canine risk factors for infection with *B. burgdorferi*.

Study Significance

As previously shown, Lyme disease is one of the most commonly reported vector-borne diseases in the United States. It can be a debilitating disease, especially if not treated in its early stages. The current passive surveillance system may underestimate the true incidence of disease. Veterinary surveillance for Lyme disease in dogs may have utility in identifying Lyme disease in non-endemic areas. Because of their close association with humans, dogs can be indicators of potential human risk.

Dogs have proven to be effective indicators of human disease in previous studies in limited geographic areas. The study of a broader geographic area, in which the vectors have established populations, is warranted. If dogs prove to be effective indicators of Lyme disease in a larger geographic area, veterinary surveillance could serve as an early detection system for the occurrence of Lyme disease in humans, especially in non-endemic areas. This would help with targeting prevention efforts in the human population. Several of the identified risk factors could provide more information on some aspects of human exposure. The identification of canine risk factors in a wide geographic area associated with

contracting Lyme disease could also enable veterinarians to target client education and vaccination efforts.

We used patients of Banfield, the Pet Hospital® during 2002 as our canine study population. Banfield is a privately owned group of veterinary hospitals that had 302 hospitals located in 37 states during 2002. Canine medical records from these hospitals are kept in a central database, creating a unique opportunity to evaluate Lyme disease in dogs from multiple states and geographic regions simultaneously.

Specific Aims

This was a two-part study with several objectives. The goal of the first part of the study was to determine if geographical distribution of Lyme disease in dogs is predictive of human cases. This was a retrospective ecologic study using national data collected by the CDC through NETSS as a source for cases of human Lyme disease. U.S. Census Bureau data was the source of human population data. The source of canine cases and population data was Banfield, The Pet Hospital®. The goal of the second part of the study was to explore the risk factors associated with Lyme disease among canine patients of Banfield, The Pet Hospital® during 2002.

The specific aims of this study were:

1. Determine the incidence of Lyme disease diagnosis among canine patients of Banfield, The Pet Hospital® during calendar year 2002.
2. Determine if an association exists between canine Lyme disease incidence, by county, for dogs diagnosed at Banfield, The Pet Hospital® and human Lyme disease incidence, as reported by CDC, in these counties during calendar year 2002.
3. Perform a case-control study to explore risk factors associated with Lyme disease diagnosis among canine patients of Banfield, The Pet Hospital® during calendar year 2002.

Materials and Methods

This was a two-part study that utilized several data sources and analysis methods. The first part of the study involved calculating and comparing human and canine incidence. This required merging human case and population data, as well as human and canine incidence data. Canine data were used in both parts of the study, as was a zip code database, which was merged with the canine data to provide location information. The second part of the study was an exploration of canine risk factors, which required aggregation of canine data and creation of new variables prior to data analysis.

Study populations

We studied human cases of Lyme disease reported to CDC through NETSS during 2002. U.S. county population statistics, for July 1, 2002, from the U.S. Census Bureau were used to determine incidence of human disease.

We studied Lyme disease diagnosed in dogs seen at Banfield hospitals during 2002. We used canine patients of Banfield hospitals during 2002 to determine incidence of canine disease.

Data cleaning and management

Human Lyme disease case counts, aggregated by county for calendar year 2002 were obtained from the CDC. Human denominator data by county as of July 1, 2002 was obtained from the U.S. Census Bureau, Population Division

in Microsoft Excel® format and used to calculate incidence per 100,000 population for calendar year 2002.

Canine data were received from Banfield, The Pet Hospital® in Microsoft Access 2000® format and consisted of eight tables as summarized Appendix 1. Each patient had a unique identification number. In addition, unique identifiers were assigned to each patient visit. Hospitals were also uniquely identified.

For each canine patient's hospital visit during 2002 the date, weight and the amount of time the dog spent outside, as reported by the owner, were captured in the ENCOUNTERS table. Demographic information on each dog, including breed, gender, birth date, home zip code, city and state of residence were captured in the DEMOGRAPH table. Lyme disease vaccination dates were recorded in the LYME_VAC table. The reason for each hospital visit was captured in the EXAM table. Laboratory findings for each patient were found in the LAB table. The MEDICAL_NOTE table contained the veterinarian's medical notes regarding each visit. Treatments, including dispensed items, for each hospital visit were included in the THERAPY table. The HOSP_SUP table contained identification numbers for each Banfield hospital, the date each opened for business and the hospital zip code. Encounter and patient identification numbers were used to link the above tables.

Microsoft Access® (http://office.microsoft.com) was the primary software program used for data management. SPSS 11.0® (http://www.spss.com) was used for data aggregation and data analysis. SAS® 8.2 (http://www.sas.com) was used for randomization and selection of controls and statistical analysis.

ArcView® (http://www.esri.com) was used for mapping. PASS® (http://ncss.com/pass.html) was used for power calculations.

Canine case definition

When a patient visited a Banfield hospital, an initial assessment was conducted and likely diagnosis entered into the database. This field contained an extensive list of possible diagnoses from which one could be selected. This data field was not always updated when the final diagnosis was confirmed. Instead, many veterinarians typed their findings in the medical notes field. This was a memo field where patient assessment, laboratory results and treatment recommendations were entered in a narrative format. Because of the unreliability of the diagnosis field, review of the medical notes of each patient visit was determined to be the most accurate way to identify cases of Lyme disease. To narrow down the number of records that would need to be manually reviewed, Banfield database managers extracted records in which the medical notes, examination and treatment fields contained key words. Patient records with "lyme" or "tick" in the medical notes, or with both "lameness" as an examination finding and "doxycycline" as a treatment were included in the MEDICAL_NOTE table. The same was done for the table containing the diagnosis, the DIAGNOSIS table.

Medical notes for each 2002 dog visit containing "lyme" (n=156,876) and "tick" (n=14,679) in the notes field were reviewed. The table containing "lyme" in the notes field was reviewed and the majority of records were visits in which

Lyme disease vaccine was administered or discussed. To identify records specific to dogs with possible Lyme disease, the notes field was searched and a flag variable created for each record that contained the words "lyme disease" (n=891), "lyme dz" (n=348), and "tick serology" (n=83).

A form was created in Access 2000® to allow review of the medical notes memo field. As records were reviewed, it became apparent that a diagnosis of Lyme disease was accomplished by several different methods. So cases were coded according to the method of diagnosis.

Other tick-borne diseases such as Ehrlichiosis (*Ehrlichia canis*) and Rocky Mountain Spotted Fever (*Rickettsia rickettsii*) can have signs and symptoms similar to those seen with Lyme disease, as seen in Table 1.

Table 1. Clinical findings of *Borrelia burgdorferi*, *Ehrlichia canis* and *Rickettsia rickettsii* infections in dogs.

Clinical Findings	*Borrelia burgdorferi*	*Ehrlichia canis*	*Rickettsia rickettsii*
Mono- or polyarthritis/lameness	Yes	Yes	Yes
Joint swelling	Yes	Yes	Yes
Fever	Yes/no	Yes	Yes
Anorexia	Yes	Yes	Yes
Fatigue	Yes	Yes	Yes
Lymphadenopathy	Yes	Yes	Yes
Cough/dyspnea	No	Yes	Yes
Peripheral edema	No	Yes	Yes
Splenomegaly	No	Yes	No
Petechiae & ecchymotic hemorrhage	No	Yes/no	Yes
Thrombocytopenia	No	Yes	Yes
Anemia	No	Yes	No

Dogs with clinical signs and symptoms of Rocky Mountain Spotted Fever or Ehrlichiosis, such as dyspnea, peripheral edema and thrombocytopenia, and/or with positive serology for these diseases were excluded from all analyses. Dogs with a history of Lyme disease with onset before 2002 were also excluded

from all analyses, as it wasn't possible to determine the date of diagnosis. Dogs diagnosed due to clinical signs consistent with Lyme disease who were lost to follow-up were excluded from all analyses as well, because it was unknown if they responded to therapy, as seen in Table 2.

Table 2. Canine Lyme disease case definitions and corresponding codes.

Code	Description
1	Dogs with clinical signs consistent with lyme disease + Western Blot test positive
2	Dogs with clinical signs consistent with lyme disease + ELISA test positive
3	Dogs with clinical signs consistent with lyme disease + response to appropriate antibiotic treatment
4	Dogs with clinical signs consistent with lyme disease with no testing or follow-up after initiation of therapy (excluded*)
5	Not a case
6	History of lyme disease in the past (excluded*)
7	Dogs with clinical signs consistent with *Ehrlichia canis* or *Rickettsia rickettsii* infection (excluded*)

***Excluded dogs were omitted from all analyses, including incidence calculation (in both numerator and denominator) and as potential controls in the case control study.**

In the same manner, medical notes containing "tick" were reviewed and records flagged that contained the key words "tick serology" (n=401), "lameness" (n=480), "doxycycline" (n=1377). All 401 records containing "tick serology" were reviewed as were records containing both "lameness" and "doxycycline" (n=28). Each record was reviewed and flagged with a diagnosis code as shown in Table 2. In addition to searching the medical notes on key words, all patient visits in which both "lameness" was an observation during the physical exam and "doxycycline' appeared as a treatment were reviewed (n=69). Finally, medical notes were reviewed for visits in which laboratory results were positive for "lyme disease titer" and "lyme disease western blot" (n=121). These records were

identified and reviewed to insure that they had been identified and ranked by previous search methods.

Part I. Human and canine incidence

Data management

Lyme disease counts provided by the CDC were aggregated by county. Banfield data contained city, state and zip code for each patient. To obtain county of residence for dogs, a zip code database containing locator information and 2002 census data for each U.S. Postal Service zip code was merged with the Banfield patient data. The resulting file contained 7786 of 914,368 (0.85%) records with invalid zip codes. The assumption was made that dogs are patients at Banfield hospitals near their residence, and the hospital zip code was used where the patient's residence zip code was invalid.

Incidence rate calculation

Human Lyme disease cases reported to CDC for calendar year 2002, aggregated by county were used as the numerator. County population estimates for April 1, 2000 to July 1, 2002, reported by the Population Division, U.S. Census Bureau, were the denominator data. Incidence per 100,000 population, by county was calculated. Three hundred fifty-five human cases in eight states (Connecticut, Massachusetts, Maine, New Jersey, New York City, Pennsylvania, Rhode Island, and Wisconsin) were not associated with a specific county and were excluded from the county incidence calculations, but were included in the total U.S. incidence calculations.

The incidence of Lyme disease diagnoses per 100,000 Banfield canine patients during 2002 was calculated for both county of residence and hospital. For incidence by county, the denominator population was canine patients of Banfield hospitals during 2002 residing in that county. The denominator population for incidence calculation, by hospital, was the canine patient population of each hospital during 2002.

Twenty-eight Banfield hospitals were open only a portion of calendar year 2002. Of these, four hospitals had dogs with a diagnosis of Lyme disease. We tried to determine if cases diagnosed in these hospitals would have otherwise been detected at nearby Banfield hospitals by reviewing records of non-cases from these hospitals. The majority of dogs had only been patients at the newly opened hospitals and not drawn from Banfield hospitals in nearby communities. So, cases would likely not been have detected at another Banfield hospital. For this reason we corrected incidence rates for hospitals not open the entire calendar year by dividing by the proportion of the year they were open.

Spatial representation

Geographic Information Systems (GIS) mapping technology was used to examine the spatial relationship between canine Lyme disease diagnoses, human Lyme disease cases and reported distribution of the tick vectors (*I. scapularis* and *I. pacificus*). ArcView® 8.3 was used for mapping.

With GIS, each set of data points sit on a separate map layer (e.g., data points for canine case residences) which can be superimposed over other map

layers containing other data (e.g., counties with human cases). This allows the visualization of spatial relationships between the data.

A database file was created that contained the distribution, by county, of *I. scapularis* and *I. pacificus* as reported by Dennis et al.[5] Counties were identified as having "reported" populations (<6 ticks and 1 life stage identified) or "established" (≥ 6 ticks or >1 life stage identified).

A database file containing canine Lyme disease cases was created. Latitude and longitude coordinates for each zip code were added to these records using an existing reference file. Zip codes of five canine cases were not found in the reference data. The patient's hospital zip code was used as the geolocator for each of these cases. The resulting file contained X (latitude) and Y (longitude) coordinates for each case.

Human Lyme disease cases, by county were identified by state and county FIPS codes. Combined state and county FIPS codes were used to link human and dog Lyme disease cases.

Map layers were created for states, counties and zip codes from existing 2001 reference files. New map layers were created by joining canine and human data to state, county and zip code map layers already created. Files were joined using the combined state and county FIPS code or by zip code where appropriate. Table 3 lists the map layers created for this project.

Table 3. Map layers used to display human & canine Lyme disease distribution maps.

Map Layers
States
Counties
Zip codes
States with Banfield hospitals
Counties containing Banfield hospitals
Banfield hospital locations
Canine LD cases
States with canine LD cases
Counties with canine LD cases
Banfield Hospitals with LD cases
Canine LD incidence by county (corrected), 2002
Counties with Human LD cases, 2002
Human LD incidence by county, 2002
Counties reporting *I. scapularis* & *I. pacificus* populations, 1998
States with any report of *I. scapularis* or *I. pacificus* populations, 1998

Linear regression analysis

Linear regression analysis was used to determine if incidence of Lyme disease, by county, among canine Banfield patients, was predictive of human incidence in these counties during 2002. The frequencies of human and canine incidence were not normally distributed. To meet the linear regression assumption that the independent variable is normally distributed, a $\log_{10}$ transformation was performed on human incidence. Since canine incidence was also measured in cases per 100,000 population, $\log_{10}$ transformation was performed on the predictor variable as well. Residuals were analyzed for the presence of outlier points that may influence the model. Linear regression analysis was conducted in SAS® version 8, via the REG procedure.

Part II. Canine risk factors associated with Lyme disease

Data management

Variables of interest were age, gender, breed, weight, time spent outside, vaccination status and elevation. Data management and categorization are detailed below.

Age

Each dog had a variable for its date of birth in the table containing demographic data. This table was exported into SPSS and checked for duplicate records that were removed. Since the analysis was for dogs seen at Banfield hospitals during calendar year 2002, records for dogs born after 12/31/2002 were identified and excluded from the analysis. The age of the dog, in years, was calculated and a new variable for age created, giving the age of the dog at the end of calendar year 2002.

Gender

Dogs are classified as intact females, spayed females, intact males or neutered males. The gender for eight control dogs was unknown and they were omitted from the analysis.

Breed

Owners provided the dog's breed at the first visit. Over 130 breeds were represented in the Banfield data. American Kennel Club (AKC) criteria were selected as the standard for breed categorization.[43] A new variable categorizing breed was created. Two other breed associations, the United Kennel Club[44] and Continental Kennel Club[45] were searched for breeds not recognized by the AKC, and these breeds were placed in one of the 8 AKC categories based on function. The categories are sporting, hound, working, terrier, toy, non-sporting, herding and miscellaneous. Mixed breeds were included in the miscellaneous group.

Weight

The dog's weight is recorded at each visit. Each dog may have had multiple visits, and therefore multiple weights recorded over time. Non-missing weights over all visits during 2002 were averaged to obtain a weight variable for analysis.

Time spent outside

The values are "indoors exclusively", "leash walk/park", "occasionally outdoors", "outdoors exclusively" and "roams freely". This variable was recoded into ordinal values 1 through 5. Multiple records for each dog were averaged and rounded to the nearest integer so that the original scale (1 – 5) was retained.

Vaccination

The recommended vaccination schedule for Lyme disease in dogs is two vaccinations, at least three weeks apart followed by an annual booster.[46] Dogs may have had multiple Lyme disease vaccinations. The table containing Lyme disease vaccination information has the date(s) of administration of Lyme disease vaccine. Records documenting vaccination for Lyme disease during calendar years 2001 and 2002 were retained, as vaccination during the previous year may also be important in predicting immunity to Lyme disease. To determine the number and frequency of vaccinations of these dogs, three new variables were created. Records were aggregated by pet identifier to create new variables for the number of Lyme disease vaccinations received during 2001 and 2002. Records were then aggregated to create a variable indicating the last date the dog was vaccinated. Another variable indicating the dog's last hospital visit was created by aggregating the data by pet identification.

A new variable was created for the date of Lyme disease diagnosis. Lyme disease vaccinations given on after the date of diagnosis were omitted from the analysis.

Selected variables from the ENCOUNTERS, DEMOGRAPH and LYME_VAC tables were merged by the pet's unique identification number to create a table containing all dogs (n=914,368) as shown in Table 4.

Table 4. DENOMINATOR table with field description and type n=914,368.

Field	Description
HOSP_ID	Unique hospital identification
PET_ID	Unique pet identification
CITY	Pet city of residence
STATE	Pet state of residence
POSTAL	Pet zip code of residence
AGE_YR	Pet age in years
GENDER	Gender
BREED	Breed, as reported by owner
BREEDCAT	Breed category
AVE_WT	Average weight
RND_ENVI	Average amount of time dogs spends outside, rounded to the nearest integer
VAC_NUM	Number of Lyme disease vaccinations

Case-control study

A matched case-control study was performed to explore risk factors associated with Lyme disease diagnosis among canine Banfield, The Pet Hospital® patients during calendar year 2002 using cases already defined (See Part I Human and canine incidence).

The number of controls needed per case was determined by conducting a power analysis. Assuming a probability of exposure in controls of 0.40 and a correlation of exposure between matched individuals of 0.20, 35 controls matched to each case achieves 80% power to detect an odds ratio of 1.62 versus the alternative of equal odds using a Chi-Square test with a 0.05 significance level. PASS® (http://ncss.com/pass.html) was used for all power calculations.

Control Selection

Control subjects were selected among dogs seen at Banfield hospitals for reasons other than Lyme disease during 2002. Dogs who were identified as cases, lost to follow-up, previously diagnosed with Lyme disease or who had clinical signs consistent with Rocky Mountain Spotted Fever or Ehrlichiosis were excluded as potential controls.

Controls were matched to cases by the Banfield hospital where they were patients. Matching on hospital was chosen to decrease the likelihood of confounding due to variations in procedures and practices that may occur between hospitals. Potential controls were placed in individual datasets by hospital. A new variable was assigned to each potential control indicating how many cases occurred in that hospital. The number of cases per hospital ranged from one to ten. Potential controls were then assigned a random number using the RANUNI function in SAS®. They were then sorted by random number and 35 selected for each case. All controls were saved in a new dataset. A new variable indicating case status was created. Since there were 144 cases, there were 144 strata with one case and 35 controls in each.

Conditional logistic regression analysis

Because multiple controls were matched to each case, the PHREG procedure in SAS® was used to fit the conditional logistic regression model. Although this procedure is designed to fit a proportional hazards (PH) regression model to survival data, the likelihood for the conditional logistic regression is

equal to the likelihood for the stratified PH model (up to a constant multiplier). Thus, estimates obtained from this procedure can be used for retrospective case control studies that employ one-to-many matching. This was accomplished by creating a stratum (hospital) for each case and its 35 controls. Within each stratum, a survival time variable was created where cases = 1 and controls = 0.[47] The distribution of each independent variable was determined. Where appropriate, the variable was categorized and simple conditional logistic regression conducted. Independent variables and categorizations are listed in Table 5.

Table 5. Categories of independent variables included in simple conditional logistic regression models.

Independent variable	Variable type	Categorization	Referent category
Age	Continuous		
	Dichotomous	≤ 2 & > 2 yrs	≤ 2 yrs
	Ordinal	> 1.5 & ≤ 3.5 yrs; > 3.5 & ≤ 7 yrs; > 7 yrs	≤ 1.5 yrs
Gender	Categorical	Female; Male; Male/neutered	Female/spayed
	Dichotomous	Male (regardless of neutered status); Female (regardless of neutered status)	Female
	Dichotomous	Intact (regardless of sex) Neutered (regardless of sex)	Intact
Breed Category	Categorical	Sporting; Hound; Working; Terrier; Non-sporting; Herding; Misc	Toy
	Categorical	Working; Terrier; Non-sporting; Misc Sporting/Hound/Herding	Toy
	Categorical	Working; Terrier; Toy; Non-sporting; Misc	Sporting/Hound/Herding
Weight	Continuous		
	Dichotomous	≤ 30 & > 30 pounds	< 30 pounds
	Ordinal	≥ 15 & ≤ 30; > 30 & ≤ 45; > 45	< 15 pounds
Time outside	Ordinal	Leash walked/park; Occasionally outdoors; Outdoors confined; Roams freely	Indoors exclusively
	Ordinal	Indoors exclusively; Outdoors confined/roams freely	Leash walked/occasionally outdoors
Vaccination	Dichotomous	Yes/no	No vaccination
	Ordinal	Vaccination w/i 365 d of last visit or dx; Vaccination greater than 365 d of last visit or dx	No vaccination
	Continuous	Number of days from last vaccination to dx (cases) or last visit (controls)	
	Ordinal	1-85 days since last vaccination; 86-209 days since last vaccination; More than 209 days since last vaccination	0 days
Elevation	Continuous	In feet	
	Dichotomous	< 200 & ≥ 200 ft	< 200 ft
	Continuous	Only for states with *I. scapularis*	
	Dichotomous	< 200 & ≥ 200 ft (Only for states with *I. scapularis*)	< 200 ft
	Continuous	Only for states with *I. pacificus*	
	Dichotomous	< 200 & ≥ 200 ft (Only for states with *I. pacificus*)	< 200 ft

Age

Age was categorized roughly by quartiles, less than 1.5 years, 1.5 to 3.5 years, 3.5 to 7 years and over 7 years. Young dogs, less than 1.5 years were selected as the referent category, as they would be expected have had less cumulative exposure than older dogs. We also dichotomized age as two years or less and over two years. Age was also analyzed as a continuous variable. The ordinal categorization by quartiles was selected for inclusion into the multivariate model because it was the most descriptive. Chi-square test for trend was conducted on estimates in the conditional logistic regression model.

Gender

Design variables were created for gender based on the original categorization with spayed females being the referent category. This group was selected as the referent because we thought that they would be least likely to roam and be lowest risk for tick exposure. They also represented the largest group of dogs in both categories. Neutered status alone was also explored as a risk factor. Dogs were also categorized based on neutered status, regardless of sex. Finally, sex alone, regardless of neutered status was explored. Combined sex and neutered status was selected as the categorization to include in the multivariate model because it was the most descriptive.

Breed

Design variables were created for the eight breed categories, with the toy breeds being the referent category. We selected this group as the referent

because toy breeds are small, generally more pampered than other breeds, and less likely to be exposed to ticks. Design variables were also created for breed categories where the sporting, herding and hound breeds were combined into one group. Due to their function, these breeds may be more likely to be outside and in areas where they could be exposed to ticks. The eight breed categorization was selected for addition into the multivariate model because it was the most descriptive.

Weight

Weights were categorized based roughly on quartiles. Small dogs, less than 15 pounds were selected as the referent category, as they would be expected to spend less time outdoors than larger dogs. We also explored weight as a continuous variable and as a dichotomous variable, less than or equal to 30 pounds and over 30 pounds. Categorization by 15-pound increments was selected as the categorization to use in the multivariate model. Chi-square test for trend was conducted on estimates in the conditional logistic regression model.

Time spent outside

The original categorization was used with "indoors exclusively" being the referent. In addition, categories "leash walked/park" and "occasionally outdoors" were combined, as were "outdoors confined" and "roams freely" to give another categorization that was explored.

Vaccination

Vaccination status was explored in several ways. As a dichotomous yes/no variable, with no record of vaccination used as the referent category. Vaccination status was also categorized by the time since last Lyme disease vaccination. Dogs with no record of vaccination were the referent category. Control dogs vaccinated within 365 days of their last hospital visit, and cases vaccinated within 365 days of their diagnosis were considered "current" on their vaccination and were grouped together. Dogs with a "lapsed" vaccination were controls and cases who were vaccinated more than 365 day of their last visit or diagnosis, respectively.

Elevation

Elevation was categorized into 200 feet or more and less than 200 feet. Analysis was performed on both the continuous and dichotomous variables. Distribution of elevation by stratum (hospital) was also evaluated. In addition, hospitals located in areas of known *I. scapularis* tick populations were compared separately from regions containing *I. pacificus*.

Multiple conditional logistic regression model

Multiple conditional logistic regression models with Lyme disease diagnosis as the dependent variable were evaluated. Assessment of confounding was conducted by assessing changes in the parameter estimates when covariates are added to the simple model. Interaction was then assessed

by Wald Chi-square test as each interaction term was added to the main effects model.

Results

Specific Aim 1. Determine the incidence of Lyme disease diagnosis among canine patients of Banfield, The Pet Hospital® during calendar year 2002.

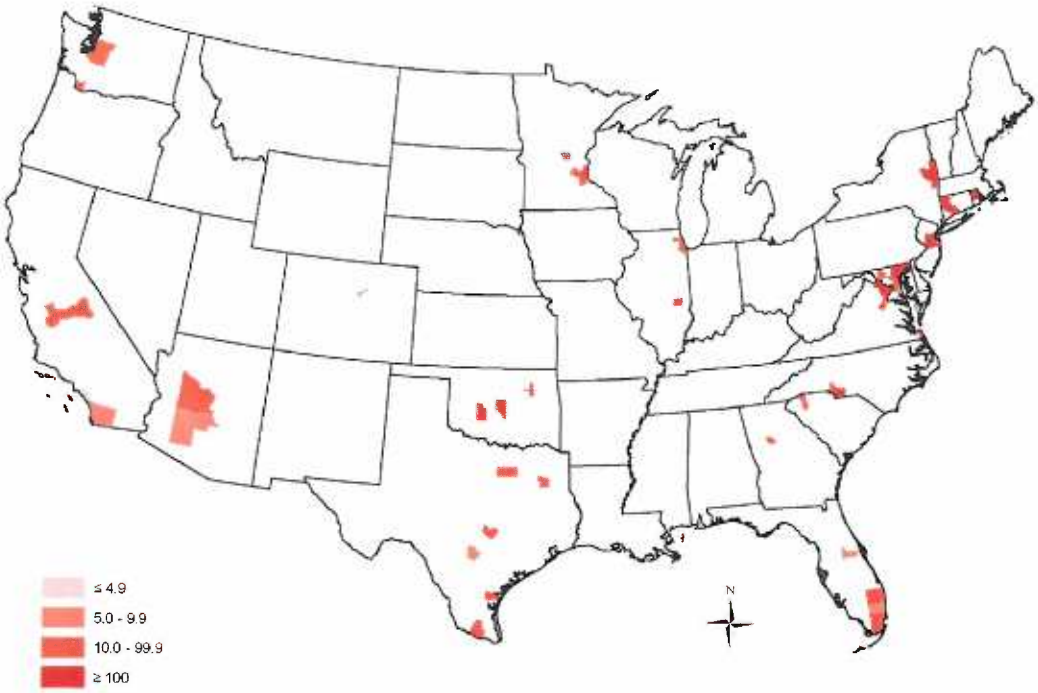
During 2002, 914,368 dogs were patients at 302 Banfield hospitals located in 37 states. Figure 1 shows hospital locations. These dogs resided in 2565 counties in 50 states and the District of Columbia. The average number of canine patients per hospital was during 2002 was 3028 with a range of 43 to 7804.

Review of 1490 canine patient records identified 144 cases. Six dogs had clinical signs consistent with Lyme disease and were western blot positive. One hundred dogs had consistent clinical signs and were ELISA positive. Thirty-eight dogs had consistent clinical signs with response to appropriate antibiotic treatment. One thousand forty-eight (70%) of records reviewed were not cases. These records contained key words because of Lyme disease vaccination or education efforts recorded in the medical notes. Ninety-one (6%) of those reviewed were dogs with clinical signs and symptoms consistent with and/or serologically positive for Rocky Mountain Spotted Fever or Ehrlichiosis. One hundred fifty-six (11%) dogs had consistent signs and symptoms, no serologic testing, treatment for Lyme disease, but were lost to further follow-up. Dogs lost to follow-up, and those with disease consistent with Rocky Mountain Spotted Fever or Ehrlichiosis were excluded from all analyses.

Cases of canine Lyme disease were diagnosed in 68 counties of residence in 20 states. Overall canine incidence of Lyme disease during 2002 in canine Banfield patients was 16.7 per 100,000 population. Figure 2 shows canine incidence, by county.

Figure 1. States containing Banfield, The Pet Hospital® and hospital locations, 2002.

Figure 2. Incidence of Lyme disease, by county, per 100,000 canine patients of Banfield, The Pet Hospital®, 2002.



Specific Aim 2. Determine if an association exists between canine Lyme disease incidence, by county, for dogs diagnosed at Banfield, the Pet Hospital® and human Lyme disease incidence, as reported by CDC, in these counties during calendar year 2002.

During 2002, 23,763 cases of human Lyme disease were reported from 783 counties in 47 states and the District of Columbia. The overall U.S. incidence during 2002 was 8.3 per 100,000 population. Figure 3 shows human incidence, by county. The northeastern and north central areas of the United States have the highest incidence rates of human Lyme disease; Connecticut having the highest at 134 per 100,000 population.

Figure 3. Human incidence of Lyme disease, by county, per 100,000 population, 2002.

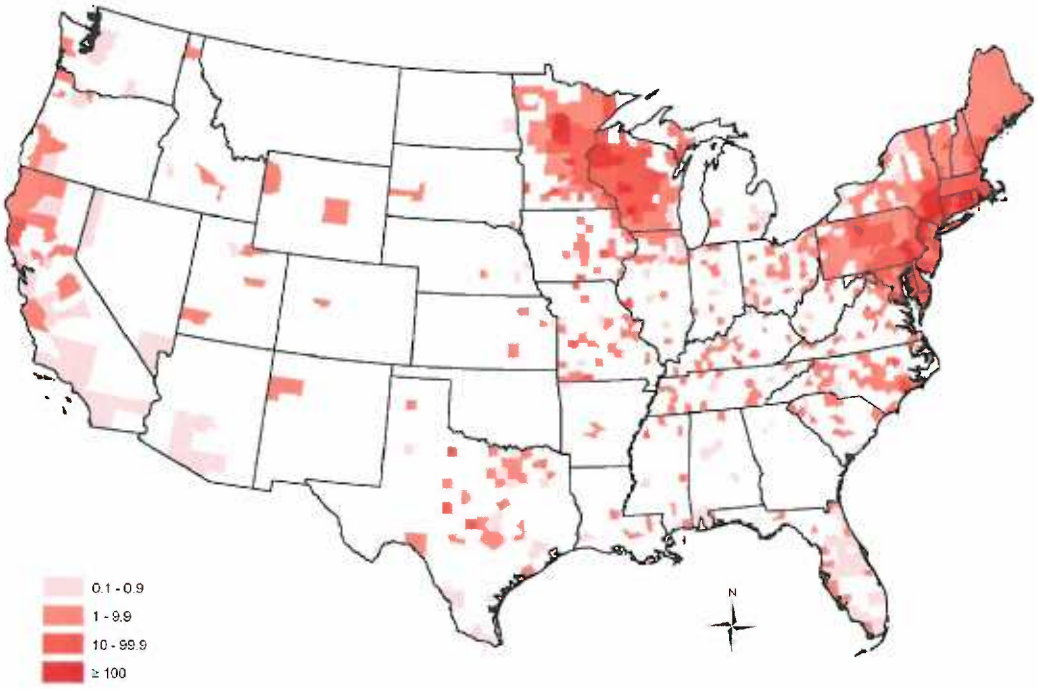
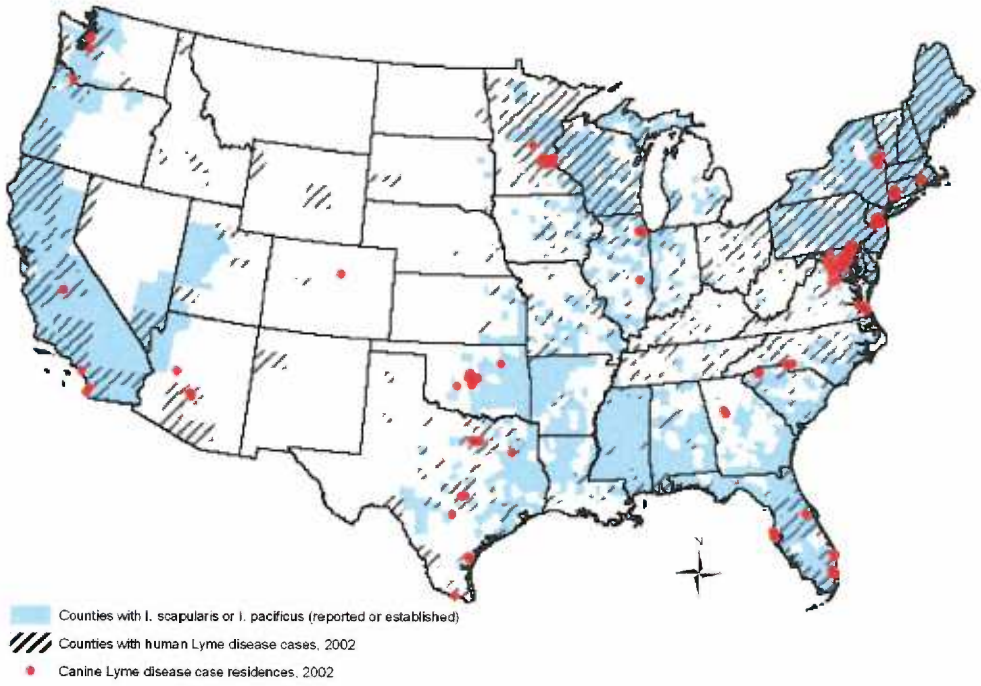


Figure 4 summarizes the distribution of the tick vectors, counties with human Lyme disease cases and locations of canine cases. Three hundred twenty-three of 783 (41%) counties reporting human Lyme disease cases had no record of the presence of the tick vector. Twenty-three of 68 (34%) counties with canine Lyme disease cases had no report of the tick vector in 1998.

Figure 4. Counties reporting human Lyme disease, tick vectors, and location of canine Lyme disease cases among patients of Banfield, The Pet Hospital®, 2002.

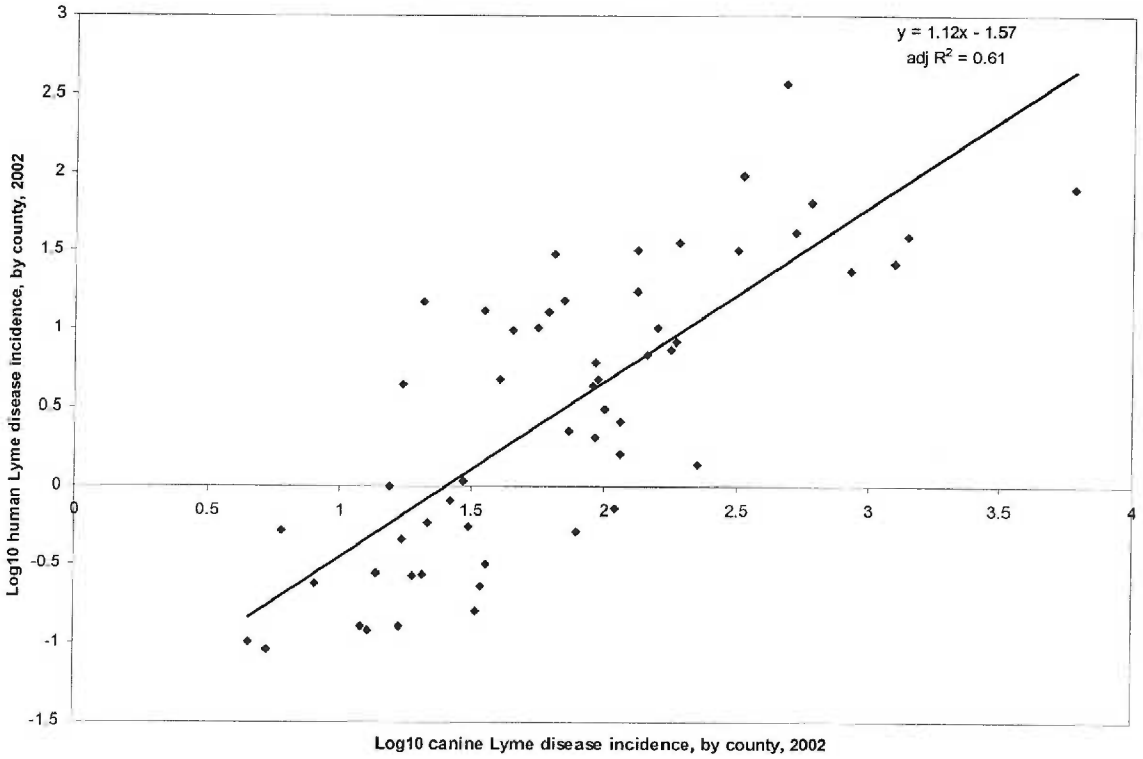


Association Between Human and Canine Lyme Disease

Fifty-seven counties contained both canine and human Lyme disease cases. Initial linear regression analysis showed that incidence of Lyme disease diagnosis, by county, among canine Banfield patients during 2002 explained 50% of the variation in human Lyme disease incidence in these counties during 2002

(Adjusted R^2 = 0.50, p-value <0.0001). Residual analysis revealed two outlier data points with substantial influence on the slope estimate. These points represented Banfield hospitals in New York and New Jersey that opened for business during 2002 with canine populations less than 50. Correcting incidence rates for hospitals not open the entire year likely resulted in an inflated incidence estimate in these hospitals. Once these two outliers were removed, incidence of Lyme disease diagnosis, by county, among canine Banfield patients during 2002 explained 61% of the variation in human Lyme disease incidence in these counties during 2002 (Adjusted R^2 = 0.61, p-value <0.0001). Figure 5 shows the scatter plot and least-squares regression line. Frequency distribution histograms of canine and human incidence and $\log_{10}$ transformations are in Appendix 2.

Figure 5. Linear regression scatter plot and least squares regression line.



Specific Aim 3. Perform a case-control study to explore risk factors associated with Lyme disease diagnosis among canine patients of Banfield, the Pet Hospital® during calendar year 2002.

Canine Study Population

One hundred forty-four dogs met the case definition for Lyme disease. Dogs who were identified as cases, lost to follow-up, previously diagnosed with Lyme disease or who had clinical signs consistent with Rocky Mountain Spotted Fever or Ehrlichiosis were excluded as potential controls, leaving 913,934 subjects. Thirty-five controls were randomly selected for each case. Table 6 summarizes the characteristics of interest for Lyme disease cases and their controls (n = 5040) from our canine study population.

Most dogs were either spayed females or neutered males. Almost all dogs were reported as being leash walked. Over 60% of dogs in the study sample had no record of being vaccinated for Lyme disease. The two breed categories most represented were the sporting and toy breeds. Twenty-one percent of the population did not have recorded weights. There also appeared to be data entry errors in some cases. With obvious data entry errors omitted, cases were, on average, 15 pounds heavier than controls.

Table 6. Characteristics of variables of interest, canine Lyme disease cases and controls, Banfield, The Pet Hospital®, 2002.

	CASES		CONTROLS	
Gender	**n = 144**		**n = 5032**	
	#	**%**	**#**	**%**
Female	10	6.9	782	15.5
Female, spayed	66	45.8	1735	34.5
Male	24	16.7	1063	21.1
Male, neutered	44	30.6	1452	28.9
Average environment	**n = 144**		**n = 5040**	
	#	**%**	**#**	**%**
Indoors exclusively	1	0.7	20	0.4
Leash walked/park	140	97.2	4921	97.6
Occasionally outdoors	2	1.4	89	1.8
Outdoors confined	1	0.7	9	0.2
Roams freely	0	0	1	0.0002
LD vaccination	**n = 144**		**n = 5040**	
	#	**%**	**#**	**%**
No vaccination	87	60.4	3203	63.6
Current (< 365 days)	56	38.9	1735	34.4
Lapsed (≥ 365 days)	1	0.7	102	2.0
Breed category	**n = 144**		**n = 5040**	
	#	**%**	**#**	**%**
Sporting	37	27.5	1132	22.5
Hound	13	9	406	8.1
Working	15	10.4	542	10.8
Terrier	17	11.8	632	12.5
Toy	12	8.3	876	17.4
Non-sporting	24	16.7	675	13.4
Herding	20	13.9	571	11.3
Miscellaneous	6	4.2	206	4.1
	Mean	**Range**	**Mean**	**Range**
Age (years)	**n = 144**		**n = 5040**	
	5.4	0.5 – 14.6	4.5	0.02 – 19.8
Weight (pounds)	**n = 143**		**n = 3947**	
	52.0	5.3 – 176	37.1	1 – 170
Elevation (feet)	**n = 141**		**n = 4999**	
	515	7 – 5368	527	4 – 5590

Simple Conditional Logistic Regression Analysis

Table 7 is a summary of the unadjusted associations of variables of interest and Lyme disease case status.

Table 7. Summary of unadjusted conditional logistic regression models.

Characteristic	Cases n (%)	Controls n (%)	OR*	95% CI for OR	Chi Square Test for linear trend
Age ≤1.5 yr	19 (13)	1335 (26)	1.00	---	
Age >1.5 & ≤3.5 yrs	38 (26)	1257 (25)	2.15	1.23, 3.75	**12.1707 p=0.0005**
Age >3.5 & ≤ 7 yrs	43 (30)	1292 (26)	2.38	1.38, 4.12	
Age >7 yrs	44 (31)	1156 (23)	2.72	1.57, 4.71	
Female, spayed	66 (46)	1735 (35)	1.00	---	
Female	10 (7)	782 (15)	0.33	0.17, 0.65	n/a
Male	24 (17)	1063 (21)	0.58	0.36, 0.94	
Male, neutered	44 (30)	1452 (29)	0.80	0.55, 1.19	
Toy breeds	12 (8)	876 (17)	1.00	---	
Sporting breeds	37 (27)	1132 (23)	2.42	1.25, 4.68	
Hound breeds	13 (9)	406 (8)	2.36	1.06, 5.22	
Working breeds	15 (10)	542 (11)	2.04	0.95, 4.40	n/a
Terrier breeds	17 (12)	632 (13)	1.97	0.94, 4.16	
Non-sport breeds	24 (16)	675 (13)	2.62	1.30, 5.30	
Herding breeds	20 (14)	571 (11)	2.59	1.25, 5.35	
Miscellaneous	6 (4)	206 (4)	2.12	0.78, 5.79	
Average weight ≤15 pounds	18 (13)	1163 (30)	1.00	---	
>15 & ≤30	21 (15)	879 (22)	1.61	0.85, 3.06	**72.1763 p<0.0001**
>30 & ≤45	21 (15)	516 (13)	2.80	1.47, 5.33	
>45	83 (58)	1390 (35)	4.26	2.52, 7.20	

*OR: odds ratio
n/a: not applicable – chi square test for trend is applicable to ordinal variables only.

Age

We found that, in the unadjusted analysis, as age increased over 1.5 years, the odds of being diagnosed with Lyme disease also increased, with dogs over 7 years of age almost 3 times more likely than dogs less than 1.5 years old to be diagnosed with Lyme disease. The increasing linear trend for the odds ratios across age categories was highly significant (Chi-square test for trend = 12.17, p=0.0005).

Gender

In the unadjusted analysis, sex without consideration of neutered status was not a significant risk factor. Neutered dogs, regardless of sex were almost two times more likely to be cases than intact dogs, when only neutered status was considered. When sex and neutered status was considered, intact females and males were significantly less likely to be cases than spayed females. This seemed unusual, so the data was reviewed for potential confounding. The proportion of cases and controls in each category were compared and no discrepancy was found.

To determine why spayed females were at more risk, an attempt was made to determine why dogs in each gender category were presented to the hospital. Diagnoses for each hospital encounter were reviewed for dogs in each gender category. Unfortunately, only 47% of case records (68 of 144 records) and 125 of 5040 (2.5%) of control records contained a presenting complaint. Diagnoses were reviewed and there was no obvious difference in reason for presentation to the hospital by gender class. When the number of encounters

for control dogs, by gender category, was reviewed, the average number of hospital visits for spayed females and neutered males was twice that of intact males and females.

Breed Category

Sporting, hound, herding and non-sporting breeds were all over two times more likely to be diagnosed with Lyme disease than toy breeds.

Weight

We found that as weight category increased, so did the odds of being diagnosed with Lyme disease, with dogs over 45 pounds being 4 times more likely than dogs less than 15 pounds to be diagnosed. For 15-pound weight categories, the Chi-square test for linear trend was highly significant (72.18, $p<0.0001$).

Amount of time spent outside

Ninety-seven percent of cases and 98% of controls were in the category "leash walked/park". There were no cases and only one control in the "roams freely" category. This variable was not explored further due to little variation between cases and controls.

Vaccination

Sixty percent of cases and 64% of controls had no record of vaccination for Lyme disease. Of those who were vaccinated, 98% of cases and 94% of

controls had "current" vaccinations (within the last year). Vaccination was not a significant risk factor.

Elevation

Few hospitals had significant differences in elevation of patient residences. Overall, elevation was not a significant risk factor. To determine if elevation was important for each tick species, hospitals located in areas of known *I. scapularis* tick populations and hospitals in regions containing *I. pacificus* were evaluated in separate conditional logistic regression models. Neither was a significant predictor of diagnosis of Lyme disease (data not shown).

Multiple conditional logistic regression model

Variables initially included in the main effects model were age, gender, breed category and weight. Breed category and weight were highly associated; so parameter estimates changed significantly when both were added to the model. Therefore, two main effects models were evaluated, either with breed category or with weight. The model containing breed category was selected as the main effects model because it is more descriptive than the model containing weight, and because 1093 records (21%) had missing values for weight. The final main effects model included gender, breed category and age.

Assessment of confounding and interaction

Covariates that appear to have a confounding effect on each other are age and gender. Parameter estimates of both change when the other is added to the univariate model. Pair-wise interactions between age, gender and breed

category were not significant, so were omitted from the final model. The final multivariate model is summarized in Table 8.

Table 8. Odds ratios for canine Lyme disease and associated risk actors from the multiple conditional logistic regression model.

Characteristic	OR*	95% CI for OR
Age ≤1.5 yrs	1.00	
Age >1.5 & ≤3.5 yrs	1.97	1.12, 3.47
Age >3.5 & ≤ 7 yrs	2.04	1.16, 3.57
Age >7 yrs	2.25	1.27, 3.96
Female, spayed	1.00	
Female	0.41	0.21, 0.81
Male	0.73	0.44, 1.19
Male, neutered	0.84	0.57, 1.24
Toy	1.00	
Sporting	2.17	1.12, 4.21
Hound	2.07	0.93, 4.60
Working	1.99	0.92, 4.31
Terrier	1.97	0.93, 4.16
Non-sport	2.29	1.13, 4.64
Herding	2.29	1.10, 4.75
Miscellaneous	1.79	0.65, 4.92

*OR: odds ratio

Spayed females had 40% the odds of being diagnosed with Lyme disease as intact females. Sporting, herding and non-sporting breeds were twice as likely to be cases as toy breeds. Dogs over 1.5 years old were twice as likely as those 1.5 years old or less to be cases.

Discussion

This is the first study to examine the diagnosis of canine Lyme disease in a population of dogs from a large geographic area. It is also the first to compare reported human and canine incidence. Previous studies have looked at seroprevalence in smaller populations in areas of the U.S. where Lyme disease is endemic,[24,27-33] and sampling and diagnostic methods varied among studies.

The overall incidence of canine Lyme disease diagnosis in our study was 16.7 cases per 100,000 canine Banfield patients during 2002. The incidence of human Lyme disease during 2002 was 8.3 per 100,000. This difference may be due to the fact that dogs are, in general, more likely to be exposed to ticks than humans, which would in turn make them more likely to contract tick-borne diseases. However, the human incidence was calculated using an estimate of the entire U.S. population for 2002 (The U.S. Census). The canine population used for incidence calculation was patients of Banfield hospitals during that year, not the U.S. canine population. The use of a hospital population relies on subjects being presented, frequently due to illness, to an animal hospital. Although Banfield hospitals stress preventive medicine and wellness programs, some patients are presented only for illness, some because of Lyme disease. So, use of the hospital population as the denominator could result in an overestimation of true incidence. Canine incidence rates in 23 states would have changed slightly in magnitude if dogs clinically diagnosed, but excluded from analysis due to loss to follow-up had been included in the analysis. An additional

five states (Kansas, Kentucky, Nevada, Ohio, and Oregon) would have been represented had these dogs been included in the analysis.

Our study showed that canine incidence accounts for 60% of the variation in human incidence. This supports several studies that evaluated canine seroprevalence as a predictor for human disease in endemic areas. Lindenmayer and colleagues found that seropositive dogs accounted for 80% of human disease averaged over a five year period in Massachusetts.[24] Johnson and colleagues also found a significant correlation ($r = 0.96$) between dog seropositivity and human cases, by county, in Rhode Island.[23] These correlations are likely to be influenced by recognition of the disease by veterinarians and physicians in an area. If human Lyme disease incidence is high, veterinarians may be more likely to look for it in dogs. If this is the case, Lyme disease diagnoses in humans and dogs are not independent of each other, resulting in an overestimation of the true correlation between canine and human incidence. The excluded cases mentioned above, if included in the analysis, may have also affected the correlation between human and canine incidence.

Unlike previous studies, we looked at incidence of the diagnosis of clinical Lyme disease diagnosis, not seroprevalence. Although canine seroprevalence is highly correlated with human disease, antibody titers can persist for years, making it difficult to determine when exposure occurred. In addition, serological tests for antibody to the organism range in sensitivity and specificity. Vaccinal titers interfere with many of these tests, making accurate diagnosis without clinical signs difficult.[18] All of these factors interfere with determining incidence of

disease, which is useful in determining trends over time. The use of newer more specific tests along with the presence of clinical disease should more accurately identify incident cases. Once these tests are widely used, further studies should be conducted on canine incidence of Lyme disease nationwide to determine disease trends.

Both human and dog cases were found in counties the tick vectors had not been reported by Dennis and colleagues in 1998.[5] Thirty-six percent (52) of canine cases were located in counties where the tick vectors had not been reported. One canine case was located in Colorado. Three of four cases in Arizona were approximately 100 miles from a county reporting the presence of *I. pacificus.* Another case, in southern Texas, was approximately 70 miles from a county reporting the presence of *I. scapularis.* Forty-one percent (323) of human cases were also located in counties where the vector had not been reported, primarily in Tennessee, Kentucky, Ohio, eastern North Carolina, Virginia, West Virginia, Northern Minnesota and sporadically in the Plains and Rocky Mountain states. One explanation could be that these dogs and people were not exposed near their residence, but in an area where the vector was present. Another explanation could be that the range of *I. scapularis,* in the eastern United States, and *I. pacificus* in the west, is expanding. The range of *I. scapularis* in the northeastern United States is increasing, and expansion of the white-tailed deer populations in this area is thought to be a factor.[5] In today's mobile world, humans and their pets may also assist in translocation of ticks as they travel or relocate. The most recent estimation of distribution of these ticks was published

in 1998. Since the existing literature on geographic distribution of tick vectors is dated, dogs with Lyme disease can serve as indicators of the presence of the tick vector in an area. Since dogs generally have more tick exposure than humans, diagnosis by an astute veterinarian could herald the possibility of human cases. Identification of ticks collected from healthy dogs can also aid in estimation of tick distribution, and expansion of populations.

Several potential risk factors for dogs were examined, but only gender, breed category and age were significant for developing Lyme disease. Sporting and herding breeds appear to be twice as likely as toy breeds to be cases, which supports the existing literature.[24,25,31] Lindenmayer and colleagues found that sporting and large mixed breed dogs were four times more likely to be seropositive than toy and small breed dogs. Guerra found that hunting and herding dogs were 1.6 times more likely to be seropositive than "pet" dogs. In a serological survey conducted in Texas, Cohn and colleagues found that the proportion of Labrador Retrievers that were seropositive was significantly more than in seronegative dogs. Due to their function, temperament, and size, these dogs likely spend more time outdoors than smaller breeds, and therefore are at more risk for tick exposure. Our study found that the non-sporting class was also at increased risk for contracting Lyme disease when compared to toy breeds. This group, which comprised 17% of cases, is not classified based on a specific function, and has a diverse range of size and coat type. When breeds of the non-sporting cases were examined, 58% were medium to large breeds (Dalmatian, Chow Chow, Shar-pei, Standard poodle and Finnish Spitz), which

also agrees with Lindenmayer's findings. Because of their function and size, other breed categories such as the hounds, working and terrier groups would also likely spend a significant amount of time outdoors, were also more likely to be cases than toy breeds, although not statistically significant. Breed category and weight were highly correlated, indicating that smaller dogs are at less risk than bigger dogs to be cases.

Ninety-seven to 98% of dog owners in our case control study said that their dogs were leash walked in the park. If this were the case, the potential for tick exposure would likely be low, due to the height and type of vegetation dogs would be exposed to being leash walked in a park. This answer may have been given because it was the option that most closely matched the true environmental exposure, since there were only five choices. In general, conscientious dog owners are expected to maintain their dogs on leashes. This expectation may have prompted owners to indicate they leash walked their dog. Accurate measure of environmental exposures would require a detailed questionnaire, completed by the owner, which may be impractical in a busy veterinary practice.

Our findings agree with several previous studies that showed older dogs were more likely to be seropositive for Lyme disease than younger dogs.[24,41] Lindenmayer found that dogs two years and older were almost three times more likely to be seropositive than dogs less than two years old. Arashima found that the proportion of dogs one year and older with antibody to *B. burgdorferi* was significantly higher than dogs less than one year old. These studies looked at

seroprevalence, not clinical disease. A large percentage of healthy dogs can have antibody to *B. burgdorferi,* which can last for years.[18] In our study, dogs over 1.5 years old were twice as likely as those 1.5 years old or less to be cases. The largest increase in risk was from 1.5 to over 1.5 years old. As dogs aged over 1.5 years old, their risk increased only slightly. The development of immunity with natural exposure to the organism, one argument against vaccination for Lyme disease, could explain our findings. Young dogs exposed to *B. burgdorferi* may develop antibody, through natural exposure, which gives them some protection from developing clinical disease as older dogs, thus explaining why risk increases only slightly in dogs once they reach 1.5 years of age. To fully address this issue, canine seroprevalence and the development of clinical Lyme disease would need to be examined prospectively.

Our study found that spayed females are more likely than intact females to contract Lyme disease. There was no difference between males and females, regardless of neutered status. This disagrees with Cohen's finding that male dogs were more likely to be seropositive than females.[31] One explanation for our findings could be that intact females are kept inside more often, due to their breeding status, reducing their exposure to ticks. Another explanation is that younger females, who have not yet been spayed, are also less likely to be exposed due to their age. Age and gender had a confounding effect on each other. There may have been some residual confounding between gender and age in the multiple regression model. Intact females represented 16% of dogs clinically diagnosed with Lyme disease, but lost to follow-up. These dogs were

excluded from the analysis. Seven percent of cases and 16% of controls in our study were intact females. The demographics of excluded dogs were more like controls than cases. Inclusion of these 156 cases, excluded due to loss to follow-up, in the analysis would have resulted in a decreased association between gender and Lyme disease diagnosis.

Our study found that vaccination for Lyme disease was not a significant factor. Vaccination for Lyme disease is controversial. The manufacturer has conducted challenge studies, and has shown immunity lasting up to one year. Greene and colleagues recommend that dogs receive routine vaccination in areas where prevalence of *B. burgdorferi* is high.[46] Others do not recommend vaccination, noting that immunity from natural exposure is present in the large percentage of dogs who are exposed, but do not develop clinical signs.[18] Additional studies should be conducted to determine if vaccination for Lyme disease is effective in areas with different disease prevalence. This would require a prospective study to follow vaccinated and unvaccinated dogs over time in areas with different disease prevalence to see if they contracted Lyme disease.

Veterinarians should be aware that dogs over 1.5 years of age are at increased risk for contracting Lyme disease than younger dogs. Sporting and herding breeds and medium to large dogs that would spend more time outside are also at more risk than the smaller toy breeds. This can help veterinarians target education efforts on tick avoidance. As in humans, prevention is accomplished by avoiding areas where ticks live, and by good tick control. There

are many effective products on the market such as amitraz containing collars, topical products such as fipronil and permethrins. Lyme disease should be in the differential diagnosis of any dog with lameness and fever regardless of their location in the U.S. We have shown that Lyme disease has been diagnosed in areas not considered to be endemic. Considering the high correlation between human and canine Lyme disease, veterinarians should work closely with their state and local health departments, both to share information on canine cases, and to follow the diagnosis of human cases.

Study Limitations

Data generated by passive surveillance systems can underestimate the true incidence of disease. Human Lyme disease is likely under-diagnosed and under-reported, so the true incidence is probably higher than current estimates. There is no surveillance system for Lyme disease in animals in the United States. The Office International des Epizooties (OIE) does not list Lyme disease as a reportable condition in animals. Since it is not a disease that would affect international trade or domestic livestock production, veterinarians are not required to report it to human or animal health officials. With no surveillance system, it is unclear what the true incidence of canine Lyme disease is in the United States. In addition, cases are identified based on their residence, which is not necessarily where the exposure occurred. In our study, travel history was not available for either study population.

Our canine study population was limited to patients of Banfield hospitals, not the general canine population of the U.S. The Banfield philosophy of pets as

family members draws owners who may care for their animals better than the general population. Since Banfield hospitals offer wellness programs that cover routine preventive care, canine patients on these wellness programs are likely to see a veterinarian more often than the general population, both for preventive care and for illness. So, Banfield patients may not represent the general canine population in the United States. Unfortunately, there is nothing comparable to the U.S. census for the dog population.

If these dogs had been included in the analysis, the association between gender and case status would have been diluted, and probably would have become insignificant. At the time our study was initiated, the most accurate method of identifying cases of Lyme disease was review of medical notes. Because of the large number of medical records, searching for key words was the most practical method to narrow down the number of records that required reading. By using this method, it is likely that misclassification of study subjects occurred. Because of differences in terms and abbreviations used by veterinarians, key word searches likely missed some diagnoses, resulting in misclassification of true cases as non-cases, leading to an underestimation of both incidence and association of risk factors. Ninety-one dogs were identified as having signs and symptoms consistent with Rocky Mountain Spotted Fever or Ehrlichiosis. Many also had positive serologic tests for these diseases. Demographic characteristics of these dogs were similar to cases, except 12% were intact females, compared with 7% of cases and 16% of controls. If these dogs had been misclassified and were really Lyme disease cases, we

exaggerated the association between gender and case status by excluding them from the analysis. One-hundred fifty-six dogs clinically diagnosed with Lyme disease, but lost to follow-up were also excluded from all analyses. These dogs were also similar to cases, except for a larger percentage of intact females than cases. Intact females represented 16% of dogs lost to follow-up compared with 7% of cases and 16% of controls. Exclusion of these dogs, if they were true cases, would also increase the association between gender and case status. Medical notes data were provided for calendar year 2002 only. So, we would have no information on dogs seen late in the year with follow-up in 2003. Only 8% of these dogs were seen in December 2002. The reason for not returning for follow-up check is unknown, but may have been because the dog recovered.

We did not have canine data from all states. Delaware, Vermont, New Hampshire and Maine, where the tick vector and Lyme disease are endemic, were not represented. Several states in the central U.S., where Lyme disease is not reported, did not have Banfield hospitals at the time of our study. This limits the ability to compare canine Banfield patient incidence to nation wide human incidence. It also limits the ability to estimate the current distribution of the tick vectors.

The tick vector data used in our study, published in 1998, was based on vector distribution estimates from many different data sources dating from 1907 to 1996 and is likely to be outdated.

Lyme disease is one of several tick borne diseases with similar clinical signs that are responsive to doxycycline. Diagnosis of a specific disease is

expensive, and although some pet owners have health insurance for their animals, it is still uncommon. So, empirically treating lame dogs with doxycycline is common practice due to financial constraints. Twenty-six percent (38) of canine cases were based on clinical signs and response to treatment. Ninety-one clinically diagnosed cases were excluded due to loss to follow-up. If these dogs were included, 65% of cases would have been diagnosed by clinical signs and symptoms only. Sixty-nine percent (100) of cases had positive ELISA tests, which can cross-react with vaccinal titers. Only 4% (6) of cases were confirmed by WB testing, which is quite expensive. With the advent of newer, more specific, in-house diagnostics that are less expensive for the client, the accuracy of diagnosis will likely improve, but doxycycline is still cheap.

In our case control study, cases were matched to controls based on the hospital where they were patients. This was done to decrease any bias due to variation in practice methods at each hospital. A problem created by matching on hospital was the inability to adequately evaluate elevation data. Eighty-two percent of cases lived within ten miles of their hospital. If cases are representative of the study population, we were actually controlling for elevation when we matched by hospital. Elevation was similar among all patients of a hospital, with few exceptions.

Conclusions

This is the first national study to examine the relationship of canine and human Lyme disease incidence, and to explore canine risk factors for disease. We found that, in our canine study population, dogs had twice the incidence of

Lyme disease diagnosis as the human population in the U.S. during 2002. Canine incidence of Lyme disease was shown to be predictive of human disease, suggesting that dogs may prove to be good indicators for the presence of *B. burgdorferi* and the tick vectors in an area. Dogs could be good indicators of expansion of tick populations to areas where they have not previously been identified. This could be particularly helpful in targeting public education about the disease and tick avoidance in these non-endemic areas. In addition, we found that sporting, herding breeds and other medium to large dogs were at higher risk for Lyme disease than small toy breeds, probably because of time spent outdoors and increased tick exposure. We also found that dogs older than 1.5 years are twice as likely to contract Lyme disease as younger dogs. These findings can help veterinarians target vaccination and education efforts on tick avoidance.

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Appendix 1. 2002 canine data, Banfield, The Pet Hospital®

Table 1. ENCOUNTERS n=2,345,035

Field	Description	Type
HOSP_ID	Unique hospital identification	Text
ENCOUNTER_ID	Encounter identification unique to each animal visit	Text
PET_ID	Unique pet identification	Text
START_DATE	Encounter start date	Date/time
END_DATE	Encounter end date	Date/time
WEIGHT	Pet's weight at each encounter	Number
OBSERVATION	Amount of time dog spends outside	Text

Table 2. DEMOGRAPH n=928,061

Field	Description	Type
HOSP_ID	Unique hospital identification	Text
PET_ID	Unique pet identification	Text
BREED	Breed, as reported by owner	Text
GENDER	Gender	Text
PET_BIRTH_DATE	Pet birth date	Date/time
PET_DEATH_DATE	Pet birth date	Date/time
PET_LAST_VISIT_DATE	Late encounter date	Date/time
POSTAL	Pet zip code of residence	Text
STATE	Pet state of residence	Text
CITY	Pet city of residence	Text

Table 3. LYME_VAC n=2,893,357

Field	Description	Type
ENCOUNTER_ID	Encounter identification unique to each animal visit	Text
PET_ID	Unique pet identification	Text
SALES_DAT	Sales date	Date/time

Table 4. EXAM n=1,304,984

Field	Description	Type
ENCOUNTER_ID	Encounter identification unique to each animal visit	Text
OBSERVATION	Observations during the physical examination	Text
OBSERVATION_VALUE	Value of observation	Text
ABNORMAL_FLAG	"NORMAL" OR "ABNORMAL" based on the observation value	Text

Table 5. LAB n=1,024,686

Field	Description	Type
ENCOUNTER_ID	Encounter identification unique to each animal visit	Text
DESCRIPTION	Description of test performed	Text
MEASURE	Result of test preformed	Text
NORMAL_FLAG	"NORMAL" OR "ABNORMAL" based on the test result	Text

Table 6. MEDICAL_NOTE "lyme" n=156,876, "tick" (MEDICAL_NOTE) OR "lameness" (EXAM) AND "doxycycline" (THERAPY) n=14,679

Field	Description	Type
HOSP_ID	Unique hospital identification	Text
PET_ID	Unique pet identification number	Text
ENCOUNTER_ID	Encounter identification unique to each animal visit	Text
MEDICAL_NOTE	Entire medical notes from the encounter	Memo

Table 7. DIAGNOSIS "lyme" n=153,982, "tick" (MEDICAL_NOTE) OR "lameness" (EXAM) AND "doxycycline" (THERAPY) n =18,477

Field	Description	Type
ENCOUNTER_ID	Encounter identification unique to each animal visit	Text
DIAGNOSIS	Description of diagnosis	Text
STATUS	Status of diagnosis	Text
DX_NUMBER	Diagnosis code	Number

Table 8. THERAPY n=1,747,490

Field	Description	Type
ENCOUNTER_ID	Encounter identification unique to each animal visit	Text
DESCRIPTION	Items invoiced during the encounter	Text

Table 9. HOSP_SUP n=388

Field	Description	Type
HOSP_ID	Unique hospital identification	Text
OPEN_DT	Date the hospital opened for business	Date/time
ZIP	Hospital zip code	Text

Appendix 2. Frequency distributions, human and canine incidence, by county and log_{10} transformations.

Figure 1. Frequency distribution, human Lyme disease incidence, by county, 2002.

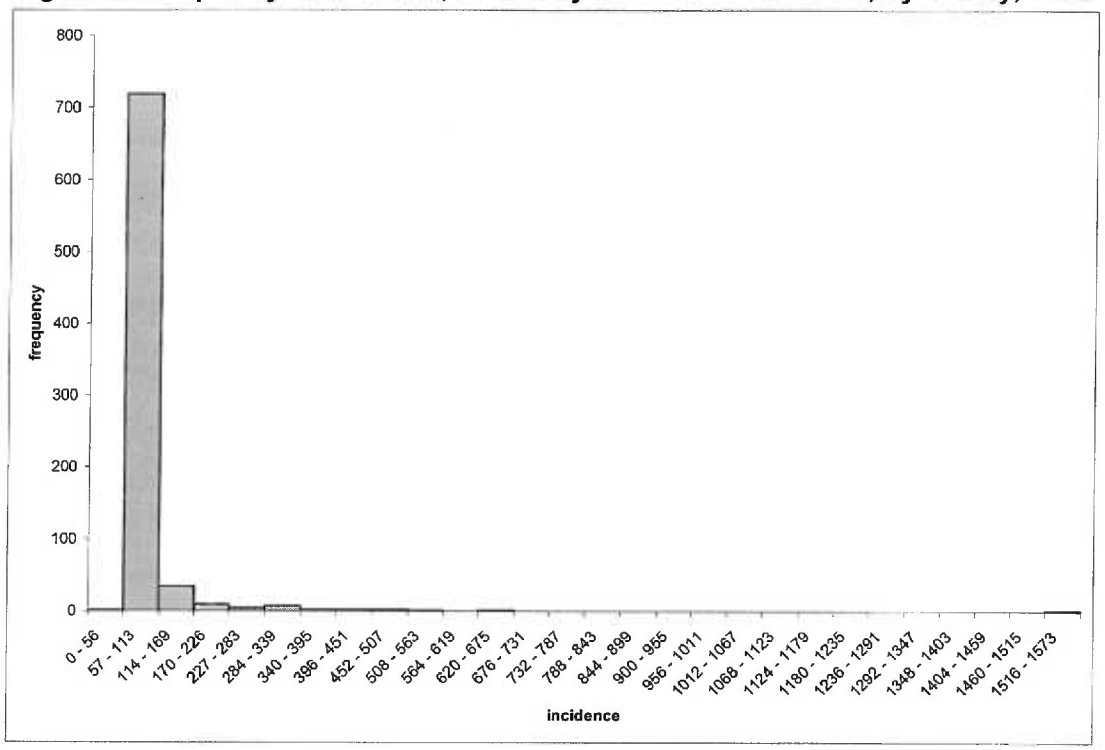


Figure 2. Frequency distribution, Log_{10} transformation of human Lyme disease incidence by county, 2002.

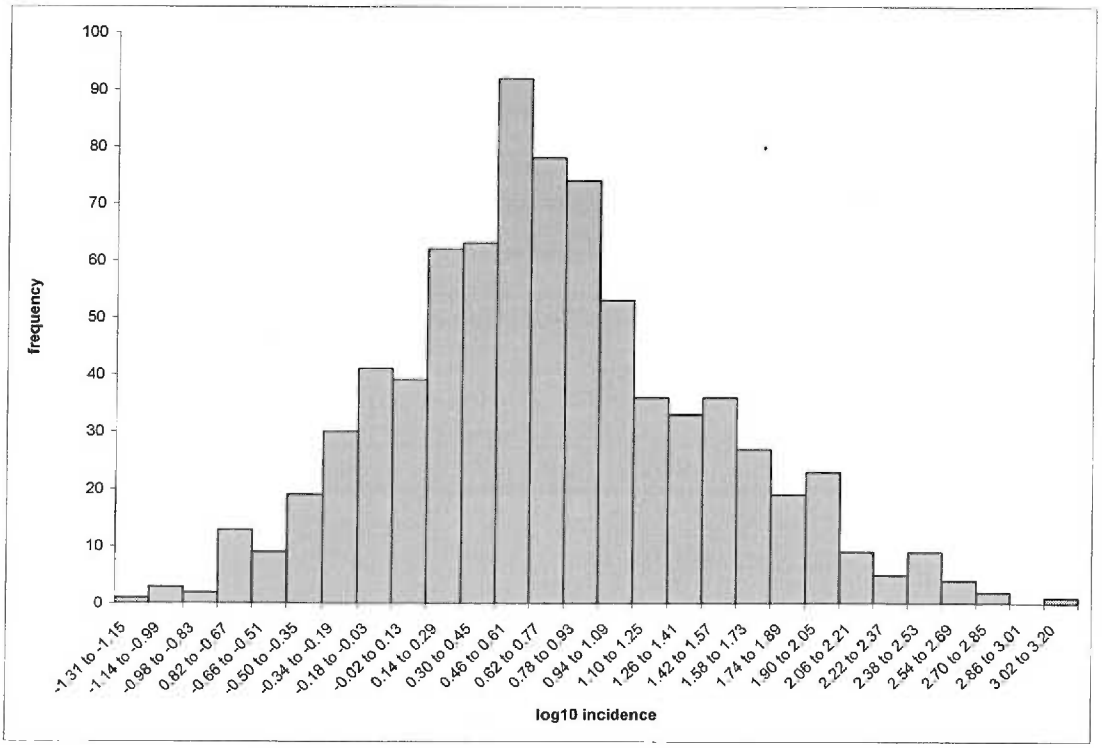


Figure 3. Frequency distribution, incidence of canine Lyme disease diagnosis, Banfield patients, 2002.

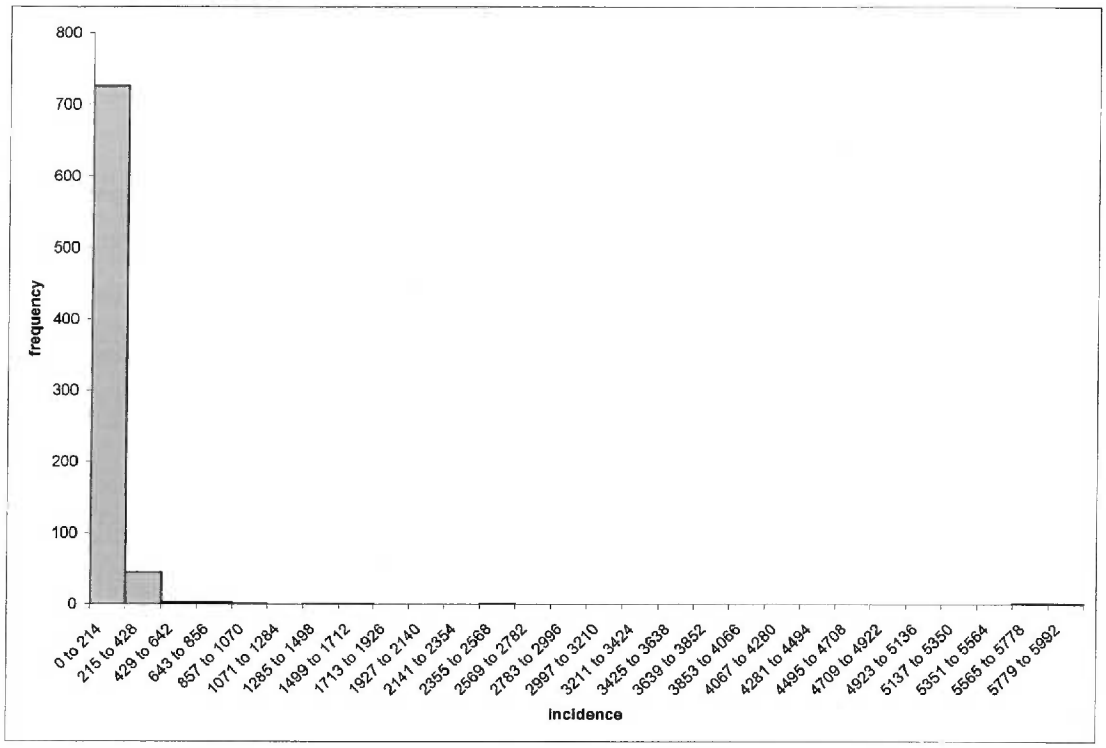
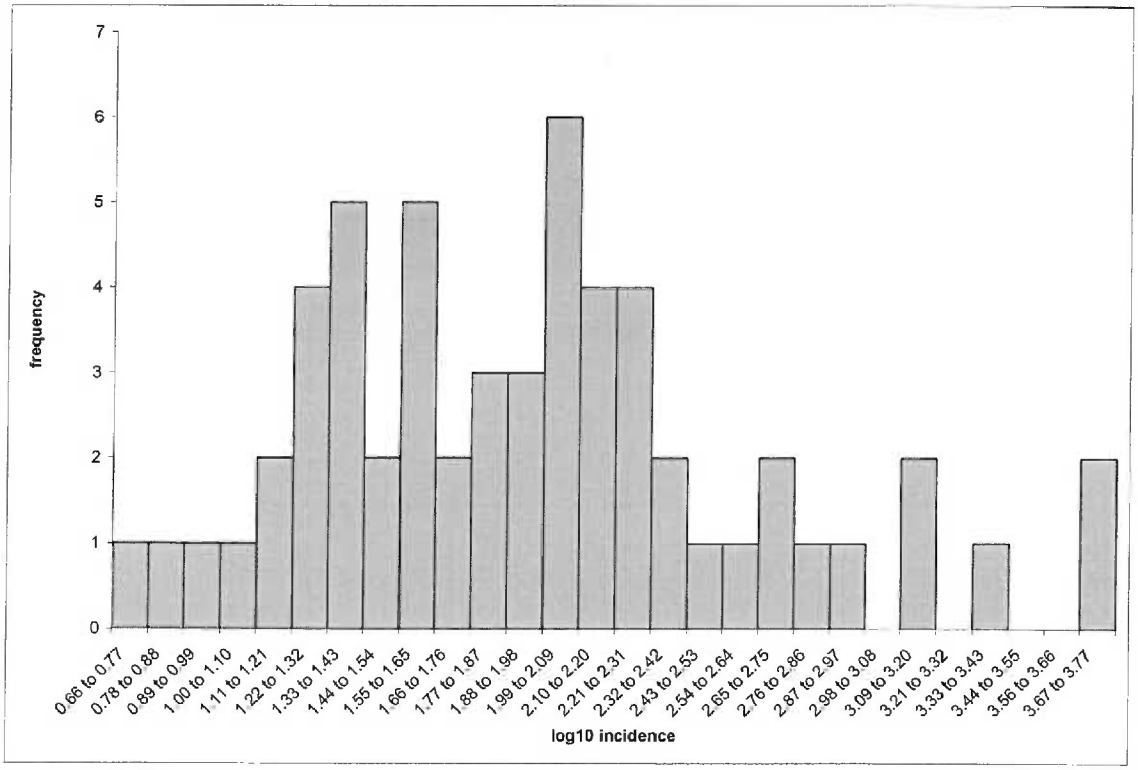
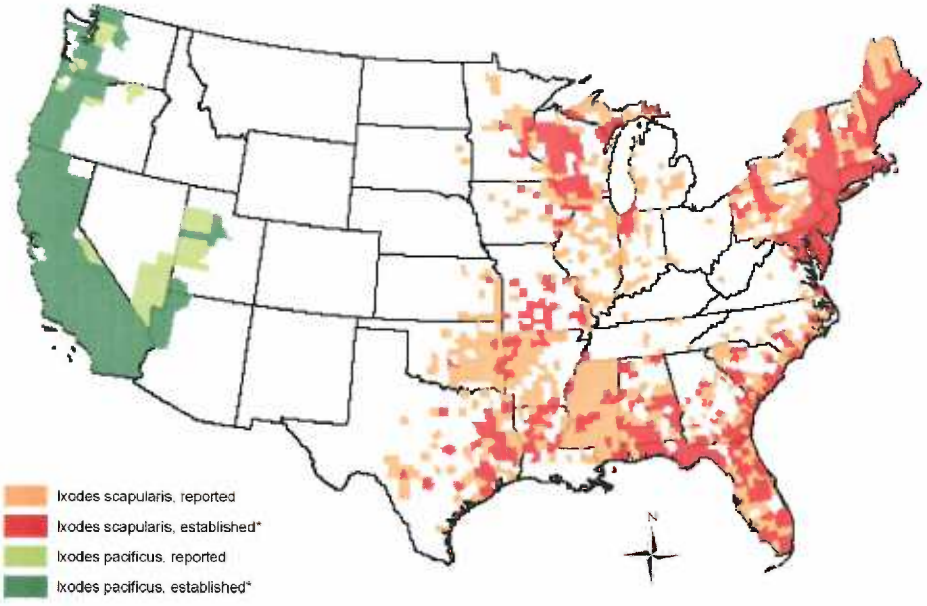


Figure 4. Frequency distribution, Log_{10} transformation of incidence of canine Lyme disease diagnosis, Banfield patients, 2002.



Appendix 3. Reported distribution of *Ixodes scapularis* and *Ixodes pacificus*, United States, 1907-1996.

Figure 1. Reported distribution by county, of *Ixodes scapularis* and *Ixodes pacificus*, United States, 1907-1996.



*Established population, at least 6 ticks or 2 life stages reported from a given county