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Environmental Health Insights

Supplementary Issue: Disease Vectors

Temporal and Spatial Distribution of Tick-Borne Disease Cases among Humans and Canines in Illinois (2000–2009)

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ABSTRACT: Four tick-borne diseases (TBDs), anaplasmosis, ehrlichiosis, Lyme disease (LD), and Rocky Mountain spotted fever (RMSF), are endemic in Illinois. The prevalence of human and canine cases of all four TBDs rose over the study period with significant differences in geographic distribution within the state. Among human cases, there were associations between cases of RMSF and LD and total forest cover, seasonal precipitation, average mean temperature, racial-ethnic groups, and gender. Estimated annual prevalence of three canine TBDs exceeded human TBD cases significantly in each region. There was concordance in the number of human and canine cases by county of residence, in annual prevalence trends, and in time of year at which they were diagnosed. To account for multiple environmental risk factors and to facilitate early diagnosis of cases, integrated surveillance systems must be developed and communication between veterinarians, physicians, and public health agencies must be improved.

KEYWORDS: human and canine tick-borne diseases, demographics, environmental risk factors, surveillance

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Introduction

Anaplasmosis, ehrlichiosis, Lyme disease (LD; borreliosis), and Rocky Mountain spotted fever (RMSF) are tick-borne bacterial diseases capable of causing significant clinical signs in several species, including humans and domestic canids. Many symptoms caused by these diseases are nonspecific, and case presentations are clinically similar. It has been reported that as many as 60%–75% of human cases are incorrectly diagnosed on the first visit to a physician after onset of symptoms.[1] All four of these diseases have been previously reported in Illinois.[2]

LD, an infection with the *Borrelia burgdorferi* bacterium, is commonly transmitted by the Ixodes spp. tick and is the most common tick-borne disease (TBD) reported in humans in North America.[3,4] Many humans infected with *B. burgdorferi* develop clinical signs, especially erythema migrans (EM) and flulike symptoms, whereas many dogs infected remain asymptomatic.[3] Dogs that do become ill often exhibit signs including polyarthritis, fever, anorexia, lymphadenopathy, and nephritis.[3,5–7]

Human and canine monocytic ehrlichiosis are caused, respectively, by *Ehrlichia chaffeensis*, which is transmitted by the Amblyomma tick genus, and *Ehrlichia canis*, which is transmitted by the Rhipicephalus tick genus. There is evidence that both humans and dogs can be infected by either ehrlichial agent.[1,8–10] Further, ehrlichial organisms may cross-react on several diagnostic tests, including an in-clinic enzyme-linked immunosorbent assay (ELISA) antigen test commonly used in veterinary medical practices to diagnose canine cases. Therefore, even though different organisms most often cause disease

in humans and canines, there is still a possibility that canine patients may serve as indicators of increased ehrlichial disease in humans.[6,11]

Anaplasma phagocytophilum, which causes granulocytic anaplasmosis, is closely related to *Ehrlichia* spp. These rickettsial bacteria are transmitted via the Ixodes genus of ticks, which also serve as vectors of *B. burgdorferi*, and infect both humans and canids.[1,6,12,13] The highest prevalence of anaplasmosis has been reported in the Midwest.[2,6] Granulocytic anaplasmosis has a similar clinical presentation as monocytic ehrlichiosis, including fever, lethargy, anorexia, lameness, and thrombocytopenia.[1,12,13]

RMSF is a potentially fatal TBD in dogs and humans, is caused by the bacterial organism *Rickettsia rickettsii*, and is transmitted by Dermacentor and Rhipicephalus spp.[6,14,15] It is considered to be the most severe rickettsial disease in the United States.[14] Signs are similar to ehrlichiosis and anaplasmosis, but petechiae and ecchymoses are more common clinically.[14] The strain infecting dogs and humans was found to be homologous, and several studies claim dogs may serve as sentinels for human disease.[14–17]

Studies have shown that ticks thrive in warm conditions with high humidity and have found correlations between the prevalence of TBDs and increasing temperatures.[18–21] The Ixodes spp. tick has been confirmed in 26 counties in Illinois and suspected to be in another 8 counties. The Dermacentor, Rhipicephalus, and Ambylomma ticks are assumed to be found in every county in Illinois (L. Haramis, PhD, email communication, July 2012). The average annual temperature in Illinois has been increasing since the early 1960s, suggesting that a broader area of Illinois may be experiencing climatic conditions ideal for ticks for a longer period of the year[22] and that the changing climate of Illinois is impacting the prevalence of TBDs.

From north to south, Illinois is almost 400 miles long and is subject to significant variations in climate, topography, forest and vegetative cover, and soil type. The state can be divided into three distinct climate zones roughly based on the National Climatic Data Center's data for mean annual temperature in Illinois.[23] To facilitate data analysis, counties were divided into the three climatic zones bordered by the Wisconsin state line and Interstate 80 (north zone), between Interstate 80 and Interstate 70 (central zone), and south of Interstate 70 (south zone) to the state boundaries (Fig. 1).

Both humans and canines are susceptible to TBDs after exposure to infected ticks. In previous studies, human and canine cases have been geographically related and it has been claimed that canine cases, because of their much higher prevalence, can serve as sentinels for human cases of LD.[24,25] A study published in 2001 described breed and exposure characteristics associated with LD in canine patients in Illinois and Wisconsin.[26] Other studies have also suggested a close temporal and spatial association between human and canine cases of TBD and a positive correlation between cases and forest cover, precipitation, humidity, and average mean temperatures.[1,22,27–30]

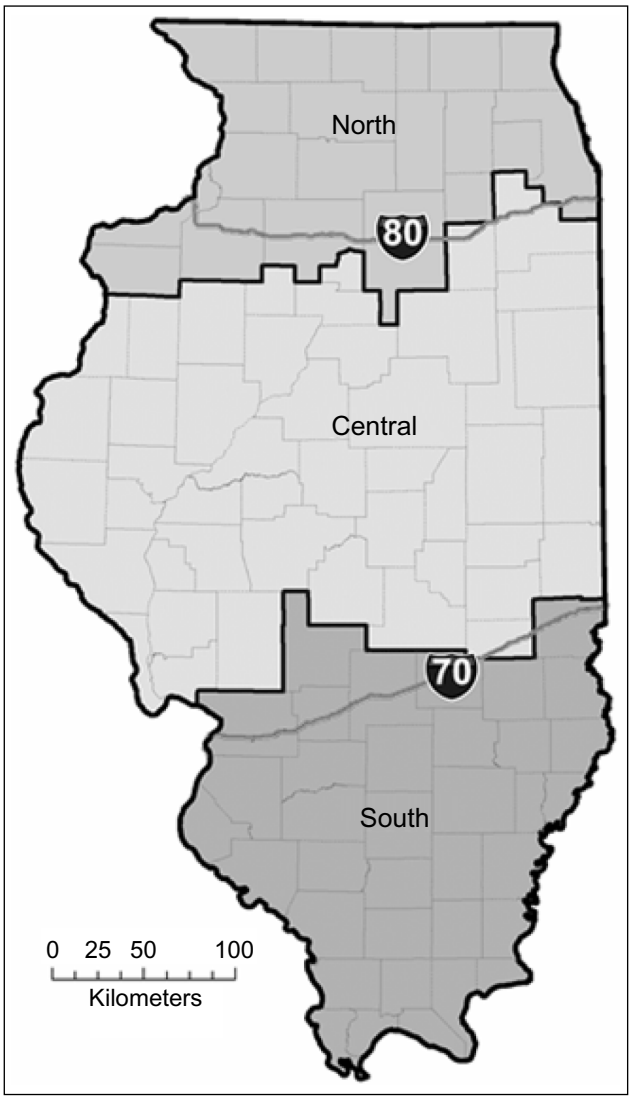

Figure 1. Climatic zones in Illinois shaded by county membership.

The number of human cases of TBDs in Illinois, as reported by the Illinois Department of Public Health (IDPH), has been increasing over the past few decades.[31] However, there is little information published on the demographic characteristics, including occupational and recreational risk factors, of human cases of TBDs.

The current report describes a retrospective cross-sectional study designed to answer five questions regarding the study period (2000–2009): (1) Has the annual prevalence of TBDs of humans and canines changed significantly in Illinois? (2) Do previously published associations between cases and environmental and weather conditions hold true in Illinois? (3) Did TBDs expand their range within Illinois? (4) Can canines be indicators of human cases of all four TBDs? (5) What are the demographic characteristics of human cases?

Methods

To assess the trends in annual prevalence, geographic distribution, the demographics of human and canine TBDs, and the effect of environmental variables on their prevalence, human and canine cases were analyzed utilizing county of residence data and categorized by the three climatic zones in Illinois. Data from the IDPH were used to determine the number of human cases of human granulocytic anaplasmosis, human monocytic ehrlichiosis, LD, and RMSF, as these diseases were reportable in Illinois from 2000 to 2009.[31] During the study period, surveillance for human TBD cases was entirely passive with case data reported by commercial laboratories directly to IDPH (C. C. Austin, DVM, PhD, email communication, September 2014). The case definitions for the human TBDs used in this analysis followed those defined by the Centers for Disease Control and Prevention and were reported as such by the IDPH (see Appendix 1). Both confirmed and probable cases were included in this survey since both are case definitions that are designated as cases under national as well as state surveillance programs.[32] Human case data were analyzed for the time of onset, age, gender, racial-ethnic group, address, year and quarter of the year, and for probable area of exposure to the infective tick.

Institutional review board approval was obtained from the University of Illinois at Urbana-Champaign (#12209). The study received a Title 45, Part 46 exemption from the IRB because all human case data were recorded and analyzed in a manner in which no human subject could be identified. To establish annual prevalence of human TBDs in Illinois, the number of human TBD cases were reported by county per year from 2000 to 2009 and divided by the population of that county on July 1st in each year. County data were aggregated into one of three climatic zones in Illinois. The annual population for Illinois counties was found using the state-based "quick facts" link on the United States Census Bureau Web site.[32]

TBDs of dogs are not reportable animal diseases in Illinois, and there is no existing database from which case information is available. A two-part survey was used to estimate the prevalence of canine TBDs during the study period and to assess demographic characteristics of and risk factors for cases. The initial survey was based on a random sample of the 727 private and corporate veterinary medical practices in Illinois, a listing of which was obtained from the Illinois State Veterinary Medical Association interactive Web site (http://isvmaimpak.networkats.com/members_online/members/directoryi.asp). An online calculator for survey sample size was used to determine a minimum sample size to achieve a 7% margin of error and a 95% confidence interval (CI) (Survey Sample Calculator; Raosoft, Inc, Seattle, WA, USA). Each practice in Illinois was assigned a random number, and practices were contacted in ascending numerical order. Practices failing to respond after two requests for information were dropped from the sample pool. Referral hospitals were included in the survey; however, cases that were referred from another practice included in the survey were cross-matched and attributed only to the referring practice.

The initial canine case survey was conducted by telephone interview, FAX, or through an email link to an online survey instrument (Surveymonkey, www.surveymonkey.com) to determine if practices had diagnosed canine TBDs during the study period according to the case definitions used in this project (Appendix 2). The initial survey took approximately 5 minutes to complete and was tested on a sample of veterinarians in academic and private practice prior to use. To estimate annual prevalence, each practice counted TBD cases for each year of the study period. Case counts were grouped by county and region and served as the numerator for prevalence rates. The total number of dogs per county per year was estimated by a human population–based formula developed by the American Veterinary Medical Association.[33] County rabies registrations were not used because, despite mandatory rabies vaccination of dogs in Illinois, not all counties require rabies vaccination registration. Responses from the initial survey identified counties in which canine TBDs had been identified and provided an estimate of statewide prevalence during the study period.

A follow-up survey was conducted with a subset of veterinary medical practices from the initial survey that were able to provide case records that held demographic and potential environmental risk factor information for cases. All practices included in the follow-up survey were able to determine, through computer-generated patient censuses or rabies tag records, the number of canine patients in the practice each year during the study period. Prevalence calculations for each practice was based on the number of cases of canine TBDs per practice per year divided by the number of canine patients per practice in each year during the study period. Once again, county data were aggregated into one of three climatic zones in Illinois. Responses from the follow-up survey estimated changes in annual prevalence rates of canine TBDs within each practice and described age, sex, breed, place of residence, exposure to geographic features, and participation in social activities such as hunting and camping.

GIS mapping and data development was carried out with ESRI ArcGIS 9.0 software (ESRI ArcGIS 9.0). We used this approach to compare county- and regional-level prevalence rates and develop summaries of temperature, precipitation, and vegetative cover that could then be assessed for an association with disease prevalence. The vegetation data were from the United States Geological Survey National Landcover Dataset from 2006 (http://www.mrlc.gov/). The climatic variables were derived from the Bioclim, Global Climate Data set (http://www.worldclim.org/bioclim). The original raster GIS data were summarized as percentages and averages for use in the statistical analysis.

Trends in the prevalence of human and canine TBDs of interest during the study period were analyzed for spatial and temporal associations by single-variable regression analysis.

Associations between prevalence and environmental factors were analyzed by chi-square analysis and multiple regression. Differences in regional prevalences were analyzed using *t*-tests and/or Multivariate Analysis of Variance. Significant associations were described at the $P \leq 0.05$ level. Data were analyzed using SPSS version 17.0 (SPSS Inc.), Statpages (http://statpages.org/ctab2X2.html), or Microsoft Excel (Microsoft, Inc.).

Results

Human cases. Due to the small number of cases of human monocytic ehrlichiosis ($n = 140$) and granulocytic anaplasmosis ($n = 28$), those cases were added together for prevalence calculations but were kept separate for geographic distribution analysis. Based on linear regression analysis of cases of TBDs by year, the average annual prevalence for human cases of LD, RMSF, and the combined anaplasmosis and ehrlichiosis cases in Illinois from 2000 to 2009 increased significantly during the study period (Table 1, Fig. 2). LD rose from 30 cases in 2000 to 138 cases in 2009. RMSF rose from 5 cases in 2000 to 110 cases in 2008 and 50 cases in 2009 and the combination of monocytic ehrlichiosis and granulocytic anaplasmosis cases rose from 1 case in 2000 to 41 cases in 2009.

Human cases of three of the TBDs showed a distinct geographic distribution (Fig. 3, Table 2). There was no statistical difference in the number of cases of anaplasmosis across the three climate zones in Illinois. However, the prevalence of LD was highest in the northern two-thirds of the state compared to the southern third, and the odds that a case of LD would occur in the northern two-thirds of the state compared to the southern third were almost four times as likely (odds ratio [OR] 3.84; $P \leq 0.000$). The highest number of cases of LD occurred in Cook County (214 cases), which includes Chicago, followed by DuPage County (97 cases), the county immediately west of Cook. The highest rates were found in Jo Daviess and Carroll counties, the two hilly, nonglaciated, and heavily wooded counties that make up the northwest corner of the state (294.4 and 94.4 cases per 100,000). Both the north and central regions showed significant annual increases in the number of cases ($r^2 = 0.806$, $P \leq 0.00004$; $r^2 = 0.7101$, $P \leq 0.002$).

The prevalence of RMSF and ehrlichiosis was highest in the southern third of the state, and the odds that cases of each would occur in the southern third of the state compared to the northern two-thirds of the state were 35.5 and 22.5 times as likely, respectively (RMSF OR 35.5, $P \leq 0.000$; ehrlichiosis OR 22.5, $P \leq 0.000$). The greatest number of RMSF and ehrlichiosis cases occurred in Jackson (32 and 17 cases) and Williamson (26 and 14 cases) counties, and the greatest rates for RMSF were found in Pope and Union counties (442.58 and 82.84 cases per 100,000K) and for ehrlichiosis in Pope and Hardin counties (93.17 and 42.95 cases per 100,000). All five counties make up the majority of the Shawnee National Forest. Both the central and southern regions showed significant annual increases in the number of cases of RMSF ($r^2 = 0.406$, $P \leq 0.048$; $r^2 = 0.45$, $P \leq 0.0033$). Only the southern region showed a significant annual increase in the number of cases of human ehrlichiosis ($r^2 = 0.833$, $P \leq 0.0002$). In addition to the overall increase in prevalence of the four

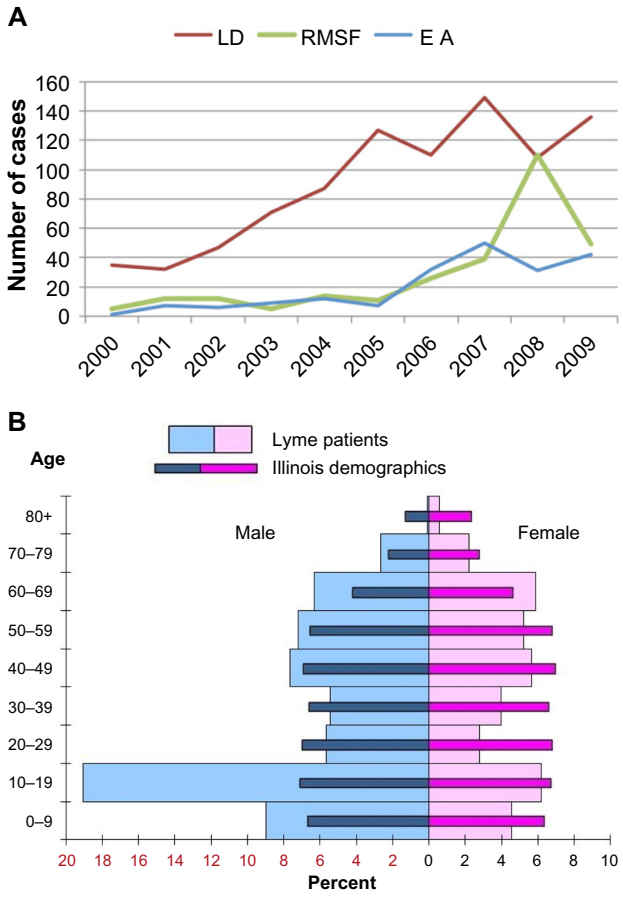

Figure 2. (**A**) Human cases of TBD reported in Illinois by year from 2000 to 2009 (LD, RMSF, ehrlichiosis, and anaplasmosis [EA]). (**B**) The age and sex distribution of reported cases of LD in Illinois from 2000 to 2009.

Table 1. Annual number of human cases of TBD in Illinois (2000–2009).

DISEASE	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	TOTAL
Ehrlichiosis and anaplasmosis	1	7	6	9	12	7	32	51	34	41[a]	200
LD	30	32	47	71	88	128	110	150	111	138[b]	905
RMSF	5	12	12	5	14	11	26	39	110	50[c]	284

Notes: [a]$r^2 = 0.749$, $P \leq 0.001$. [b]$r^2 = 0.826$, $P \leq 0.0001$. [c]$r^2 = 0.529$, $P \leq 0.017$.

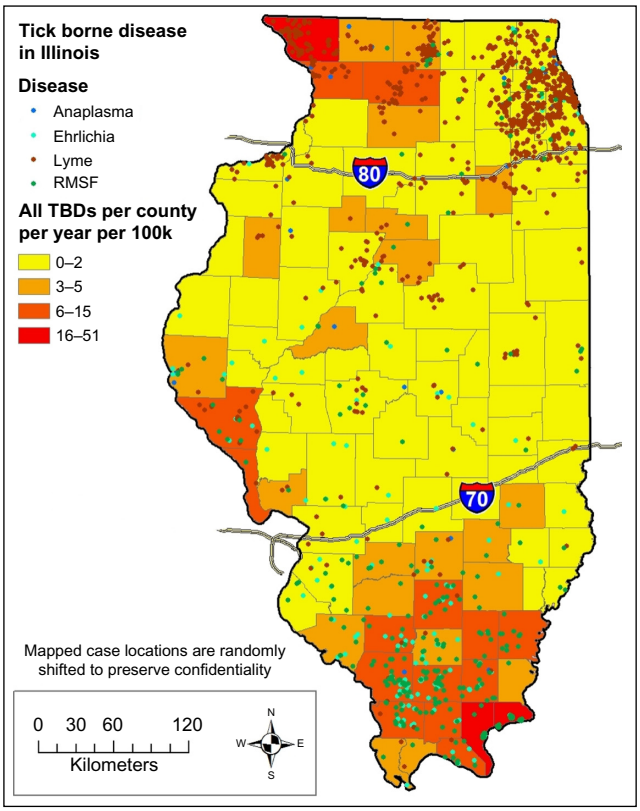

Figure 3. Geographic distribution of human TBD in Illinois (2000–2009) and the rate of TBD cases by county (reported cases/100,000 population).

TBDs in humans over the study period, LD and RMSF cases appeared to consolidate their range over the study period as evidenced by shifts in the mean center and the standard deviation ellipse of the county-level prevalence (Figs. 4 and 5).

Of the 1389 human cases of TBDs reported in Illinois from 2000 to 2009, 63% were male and the mean and median ages were 43 years, with a range of 2–88 years (Fig. 2). Throughout the state, LD cases showed a bimodal age distribution with most cases occurring in persons younger than 20 and older than 40 years of age. Of all cases of TBDs for which racial-ethnic group was listed, over 97.3% of cases were white, non-Hispanic.

Table 2. Geographic distribution of human case rates of TBD in Illinois (2000–2009; reported cases/100,000 population).

	NORTH	CENTRAL	SOUTH
Anaplasmosis	0.19	0.31	0.24
Ehrlichiosis	0.15	1.0	7.9[a]
LD	7.9[b]	6.7[b]	2.0
RMSF	0.25	1.3	17.8[c]

Notes: [a]Odds that a case of ehrlichiosis was diagnosed in the south zone compared to the north and central zones was 22.5 (15.4–33.0); $P \leq 0.000$. [b]Odds that a case of LD was diagnosed in the north and central zones compared to the south was 3.84 (2.55–5.84); $P \leq 0.000$. [c]Odds that a case of RMSF was diagnosed in the south zone compared to the north and central zones was 35.5 (26.3–48.0); $P \leq 0.000$.

Although non-Hispanic white people make up the majority of the state's population (78%), the odds that a vector-borne disease case were a white person was 10.4 times greater than for the nonwhite population (CI 7.36–14.5, $P \leq 0.000$). Additionally, the odds that a human case of a TBD disease would be a white male were more than twice as greater than other demographic groups (OR = 2.84 [2.23–2.77]; $P \leq 0.000$). The vast majority (89%) of cases of TBDs in humans during the study period were diagnosed during the second and third quarters of the year (April through September).

There was a strong association between prevalence of both LD ($P \leq 0.017$) and RMSF ($P \leq 0.001$) cases and total deciduous and coniferous forest cover consistent with their reported distributions (Fig. 6). Additionally, we examined correlations between human LD and RMSF cases and 30-year averages for temperature and precipitation, summarized by those counties in which LD (66 counties) and RMSF (58 counties) were diagnosed. There was a positive association between prevalence of RMSF and average precipitation from the months of October through May and a negative association with precipitation levels during the months of June through September during the 10-year study period ($P \leq 0.0001$). The correlation between RMSF cases and the total 30-year precipitation was also significant at $P \leq 0.000$. Interestingly, this association was the opposite of that for cases of LD for which there was a negative association between precipitation and case prevalence during the months of October through May and a positive association with precipitation during the months of June through September ($P \leq 0.034$). The negative correlation between LD cases and the total 10-year precipitation was also significant ($P \leq 0.024$). Due to the small number of human cases, we were unable to develop meaningful associations between precipitation and prevalence of anaplasmosis or ehrlichiosis.

For cases of RMSF, data analysis suggested that there was a positive association between prevalence across the state and average temperature during the 10-year study period ($P \leq 0.006$). For LD, case prevalence was negatively associated with temperature throughout the 10-year study across the state ($P \leq 0.021$)

Canine cases. Of the 727 private veterinary medical practices in Illinois, 353 randomly selected practices were contacted for the initial survey. Two hundred and seventy-nine practices (79%) responded, and 74 practices did not respond after two contacts. The distribution of responding and nonresponding practices was not related to region. With a sample size of 279 practices, the margin of error was 4.63% and the confidence level was 95%.

From 2000 through mid-2002, the most common diagnostic methods for canine TBDs were the detection of characteristic morulae in the cytoplasm of white blood cells (anaplasmosis and ehrlichiosis); polymerase chain reaction (PCR) on serum and the demonstration of a fourfold change in indirect fluorescent serum antibody titers (anaplasmosis, ehrlichiosis, LD, and RMSF); or Western blot test for LD.

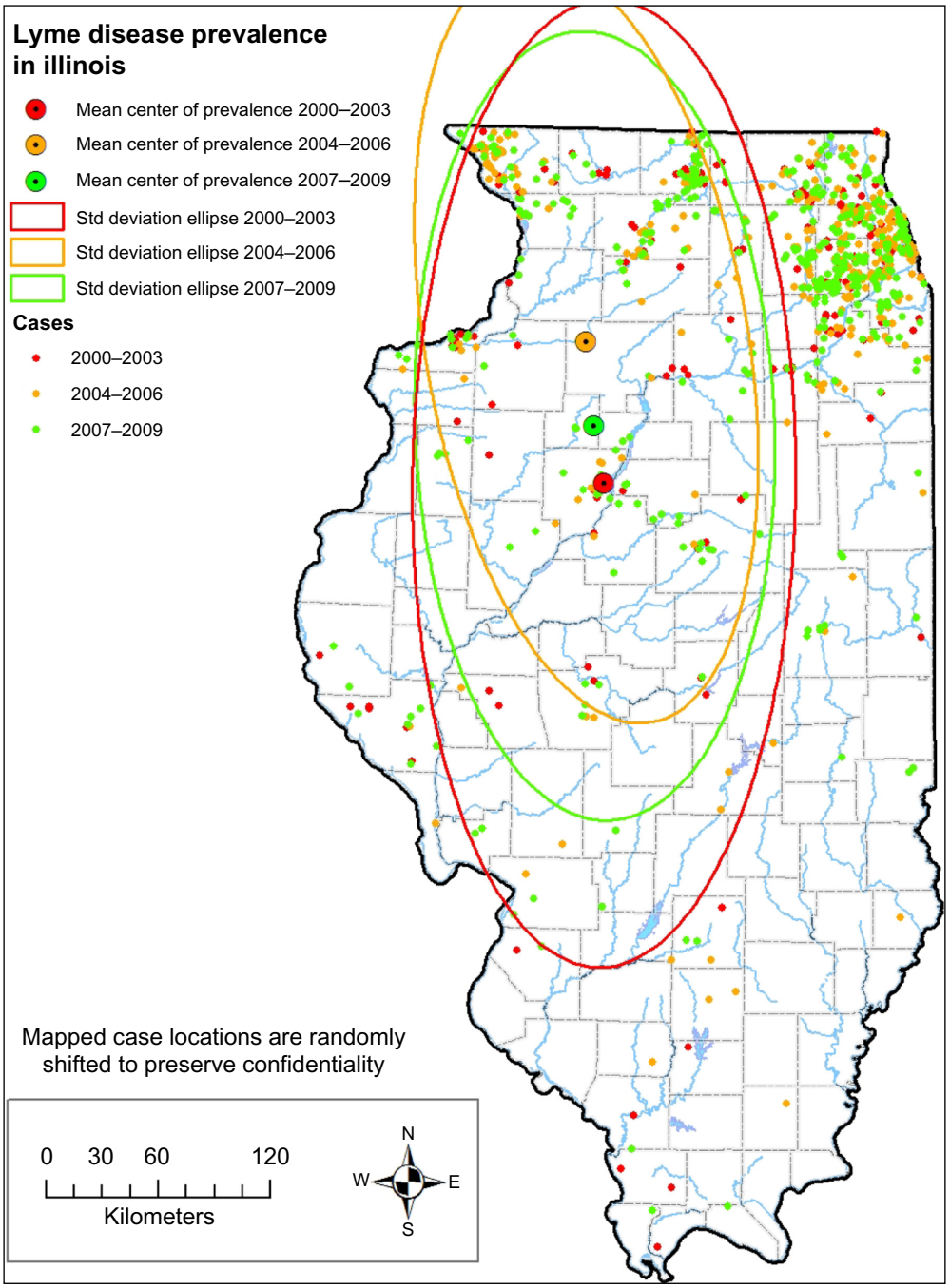

Figure 4. Change in the range of human cases of LD in Illinois (2000–2009).

With the introduction of the sensitive and highly specific in-clinic ELISA diagnostic kits in 2001 (SNAP 3Dx) and 2006 (SNAP 4Dx) (IDEXX Laboratories), the number of diagnostic testing increased considerably in veterinary medical practices.[6,11]

Of the sampled practices, 77.8% (217) diagnosed at least one of the TBDs according to the case definitions during the study period. The percentage of practices with at least one diagnosis of canine TBDs varied by region with the odds of a case being diagnosed by a northern and central region practice being 10.3 times (4.5–23.7) and 8.7 times (3.8–20.4) greater, respectively, than a case being diagnosed by a southern practice ($P \leq 0.000$). A total of 24 of 122 practices sampled in the northern region, 24 of 108 practices sampled in the central region, and 14 of 42 practices sampled in the southern region did not diagnose any TBDs from 2000 to 2009 (Fig. 7).

The estimated prevalence of the four TBDs using responses from the initial survey suggested that canines are more often diagnosed with all four TBDs than humans and diagnosed with RMSF at similar rates as humans in Illinois. However, the estimated annual prevalence of canine anaplasmosis, ehrlichiosis, and LD in Illinois was significantly less

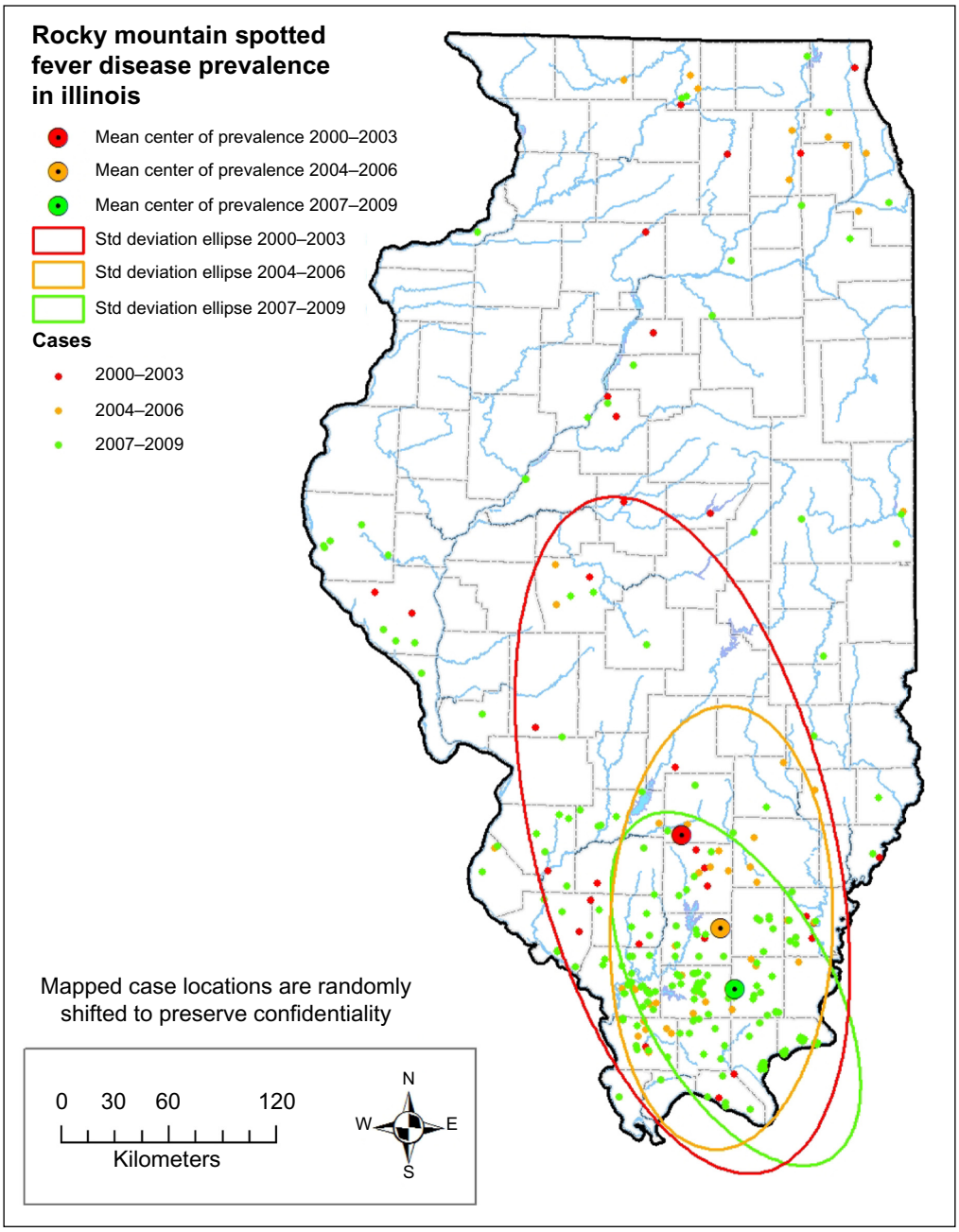

Figure 5. Change in the range of human cases of RMSF in Illinois (2000–2009).

than previously reported for Illinois in a national serologic survey of stored canine samples, which calculated a 6-year cumulative prevalence[6] (Table 3). All four canine TBD were significantly more prevalent in the south zone compared to the north and central zones, despite a lower percentage of private practices diagnosing TBDs (Table 4).

Of the practices that responded to the initial survey, 52 (18.6%) had records for 167 cases that were complete enough to assess demographic and environmental risk factors through completion of a follow-up survey. The vast majority of practices surveyed reported that, due to the ease of use, low cost, and availability of the in-clinic ELISA test cassettes, the number of tests for TBD increased annually during the study period. However, the prevalence of canine cases of TBDs could only be estimated from the records submitted by the 52 practices that completed the follow-up survey. Although we could count the number of cases diagnosed in each year of the study period and divide them by the number of test kits used annually and could estimate the number of canine patients in the practice in the later years of the study period to estimate overall annual prevalence, records were too incomplete to do so in the early years of the study period. Without this consistent denominator information, annual prevalence and trends in diagnosis were impossible to calculate. However, practices reported anecdotally that the number of cases of RMSF in the northern region and the number of LD cases in the southern

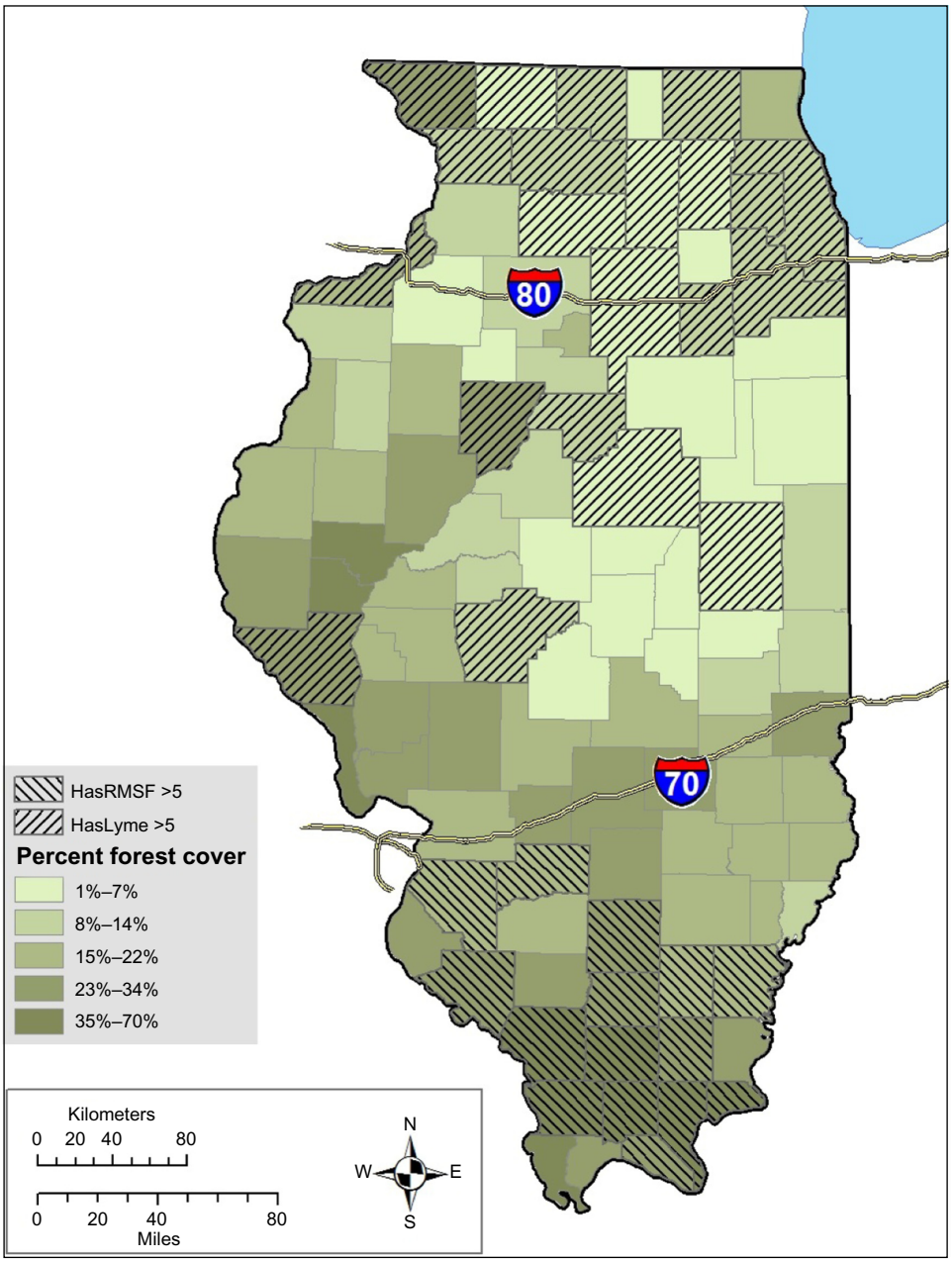

Figure 6. Counties in Illinois with more than five reported cases of RMSF or LD during the period from 2000 to 2009 and percentage of forest cover by county.

region seemed to be increasing as a percentage of tests run. There was concordance in the number of human and canine cases by county of residence, in annual prevalence trends and in time of year for diagnoses. Although many canine cases based on previous studies are believed to be asymptomatic, the similarities of human and canine case distribution and prevalence trends reported above provide evidence that canine cases can be used as indicators of human exposure.

Consistent with previous work, most of the canine cases were middle-aged (average age 5.5 years), hunting breeds (n = 127, 76%), and most were diagnosed during the second and third quarters of the year (n = 133, 79.6%). Most cases were exposed at home (n = 164, 98.3%), and almost half of the cases lived in rural areas (population of 2500 or less, n = 81, 48.5%), with 37.1% (n = 62) living in suburban areas (population 2500–10,000), and 14.4% (n = 24) in urban areas. Cases were evenly distributed by sex. Most dogs (n = 147, 88%) were either not treated with flea and tick preventative or only occasionally treated.

Discussion

Since 92 of 102 counties in Illinois reported at least one human case of one of the four TBDs in the 10-year period of the survey, it is apparent that the vectors and the pathogens of these diseases are endemic in Illinois. Some human cases, especially of LD, reported suspected tick exposures out

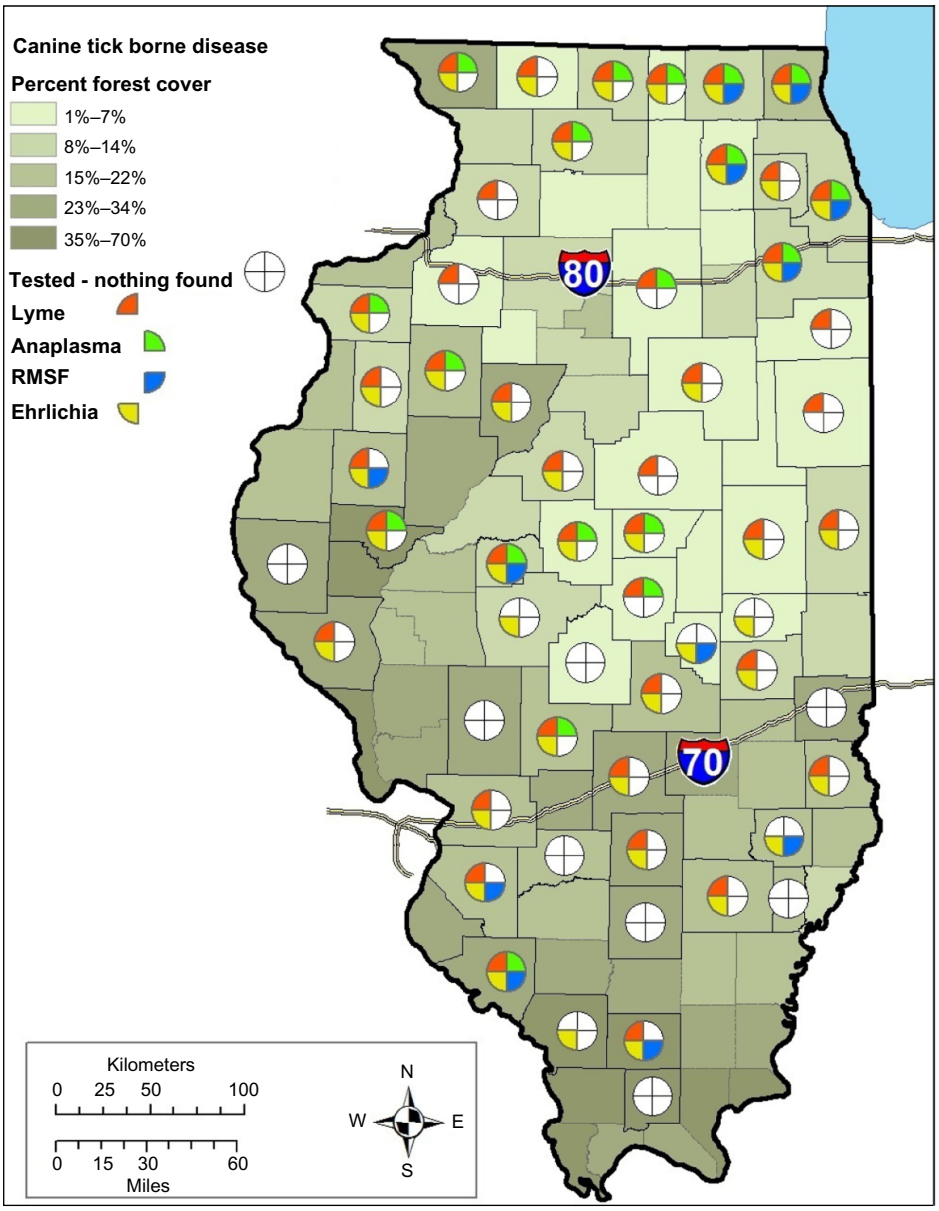

Figure 7. Canine TBD by county in Illinois (2000–2009) diagnosed by veterinary medical practices that responded to a survey initiated by the authors.

Table 3. Annual prevalence for human and canine TBDs in Illinois compared to a 6-year cumulative prevalence for canines in Illinois from a national survey.

	ILLINOIS CANINE TBD SURVEY (2000–2009) (ESTIMATED AVERAGE ANNUAL PREVALENCE)	ILLINOIS HUMAN TBD SURVEY (2000–2009) (AVERAGE ANNUAL PREVALENCE)	NATIONAL CANINE SURVEY (2001–2006) (ESTIMATED AVERAGE ANNUAL PREVALENCE)
Anaplasmosis	164/1M	0.22/1M	713/1M
Ehrlichiosis	416/1M	1.1/1M	713/1M
LD (Borreliosis)	937/1M	7.01/1M	1689/1M
RMSF	452/1M	2.25/1M	NA

of state. Although tick and pathogen exposure could have occurred outside of the state of Illinois, there is no way of knowing if that suspected exposure led to TBD or whether exposure to resident ticks within Illinois led to disease. However, all cases reporting potential tick exposure out of state lived in counties or adjacent to counties within Illinois in which the TBD with which they were affected had been previously reported.

Human cases of LD and RMSF were strongly associated with demographic, geographic, environmental, and weather factors. The majority of cases of TBDs occurred in middle-aged, white males. This distribution may reflect an interaction of exposures and access to health care. Perhaps this demographic group engages more frequently in outdoor activities, such as hunting and fishing, especially in areas of heavy vegetation, than other groups and uses protective clothing or

Table 4. Geographic prevalence of canine cases of TBD in Illinois (2000–2009; estimated cases/100,000 canines).

	NORTH	CENTRAL	SOUTH	STATE
Anaplasmosis	19.48	11.63	48.36[a]	16.38
Ehrlichiosis	20.72	27.96	218.51[a]	41.58
LD	83.82	81.41	189.85[a]	93.68
RMSF	3.58	2.23	16.12[a]	4.52

Note: [a]South zone prevalence significantly different from north and central zones; $P = 0.000$.

topical insecticides less. An assumption that genetic factors may be involved in an apparent increased susceptibility of white males to TBDs may be made, but there is not enough evidence from the current analysis to support that contention. Ultimately, the apparent increase in susceptibility of some racial-ethnic groups to contracting TBDs may be due to the interplay between individual resistance and multiple social, biological, chemical, and physical exposures.

LD and RMSF cases in the human population varied significantly by geography, with the majority of cases of LD in the northern two-thirds of the state and the majority of RMSF cases in the southern third. As one of the objectives of the current study, we wanted to determine if the range of TBDs in Illinois expanded consistently with an increase in temperature. Instead, we found that the human cases of LD and RMSF seemed to consolidate their ranges over the study period. This could be because of the interplay of tick life cycles and annual fluctuations in weather or it could be due to increased patient and provider awareness leading to an increase in testing in those areas of the state that have been traditional high–TBD prevalence areas (Figs. 4 and 5).

RMSF cases were correlated with wetter conditions especially from October through May and warmer average mean temperatures, whereas LD cases were correlated with drier conditions during those same months and overall cooler average mean temperatures during the study period. Although we did not have enough cases to analyze, it would have been interesting to investigate if cases of human anaplasmosis, which is carried by the same genus of ticks as LD, were also associated with cooler and drier weather conditions.

During the 10-year period covered by the survey, 85 of the 102 counties in Illinois reported both human and canine TBD cases and only 5 counties reported neither. In seven counties, human cases were diagnosed but no canine cases were diagnosed. In five counties, the converse was true. If cases of canine vector-borne diseases are indicators that humans are at risk for these diseases, then there should be few counties in endemic areas with human cases and no canine cases and vice versa. A diagnosis of either a human or a canine TBD indicates that, at some time during the period covered by the survey, environmental conditions were adequate to support the development of the tick vectors necessary to transmit the pathogen diagnosed, that exposure of humans or canines was sufficient to cause clinical disease, and that local human and veterinary healthcare providers should be considering this diagnosis in their patients with appropriate clinical presentations.

The human data analyzed in this report are subject to a number of limitations. First, data retrieved from the IDPH reports only those individuals who were confirmed or probable cases based on the case definition. Since tests for the four vector-borne diseases of interest are run in a number of private laboratories and not at IDPH laboratories, denominator information, ie, the total number of positive and negative tests run annually, was not available from private laboratories despite multiple requests from the researchers. We could not discount the possibility that any increases in the number of cases of each of the TBDs could have been due simply to increased annual surveillance during the study period. Second, exposure to infected tick could have occurred out of state. However, the number of patients who mentioned this possibility was few and all lived in counties or next to counties in which TBD was common. Third, the number of cases could be grossly underestimated due to the nonspecific nature of the symptoms and perhaps to issues in access to health care. Finally, the climatic data, average mean temperature and precipitation, may not be sensitive enough to link to changes in TBD prevalence over the relatively short 10-year study period.

This is the first report on the burden of all four canine TBDs in Illinois. Our data suggest that dogs may be considered as sentinels for not only human LD cases but the other three TBDs as well. Yet, our attempt to estimate prevalence rates and to correlate them with human cases as well as risk factors over the study period is subject to a number of limitations. Since TBDs are not reportable animal diseases in Illinois, there is no statewide or local registry for case reporting. Second, the total number of cases reported by many of the practice respondents in the initial survey could have been significantly underreported since a surprising number of private veterinary medical practices, whether urban, suburban, or rural, did not have computerized records and billing systems and practitioners and staff could only verify cases that were recalled by staff and for which they had paper- or computer-based records. Third, the prevalence for canine cases was based on antibody levels to TBD in a sample of dogs brought to an animal health facility because they were clinically ill and could have missed those animals that harbored inapparent or mild symptoms and therefore could have underestimated overall prevalence.

Fourth, there is no formalized canine census in Illinois. We attempted to use canine rabies immunization records as the basis of calculating canine populations per county but found that, although rabies immunization of canines is required by state law, not all of the 102 counties in Illinois require registration with a county agency. Finally, the formula used to calculate canine census in a community, well researched and documented by the American Veterinary

Medical Association, is still an estimate. In effect, both the numerator (cases) and the denominator (canine population) used to determine canine prevalence rates from initial survey data were based on estimates.

The estimated prevalence of canine TBDs in Illinois from the initial survey was significantly higher than that for humans, although much lower than previously reported prevalence.[6] In that earlier study, prevalence was calculated from the results of tests sent in by veterinarians, who were participating in a program sponsored by the manufacturer of the test kit used (IDEXX), and then extrapolated to an estimated average annual prevalence. This extrapolation may have introduced rounding errors resulting in artificially high prevalence. Also, in our study, especially during the first years of the study before widespread adoption of the IDEXX test kits, more specific methods were often used to diagnose TBDs including Western blot and PCR and could have underestimated the true prevalence of TBDs in their canine patients.

In contrast to the human data, the prevalence of all four canine TBDs was significantly higher in the south zone than in the other two zones. This was surprising because the proportion of veterinary medical practices in the southern zone that diagnosed TBDs was less than the other two zones. However, those practices that did diagnose TBDs in the southern zone could have had very aggressive testing programs and, due to the relatively small number of dogs in that zone, have skewed upward the overall prevalence of TBDs. An additional explanation, which is consistent with our canine survey findings, may be that dogs in the heavily populated northern part of the state do not send as much time outside as dogs in the more rural part of the state.

Consistent with the statewide human case data, there was an increase in the annual prevalence rate of canine TBDs reported by the subset of practices responding to the follow-up survey over the study period. More detailed information regarding the breeds of dogs infected with TBD was available from the follow-up survey. Although hunting breeds had the highest number of cases and were significantly more represented in the case data, it was impossible to assess breed-related risks for TBD infection without knowing the absolute numbers of each breed in Illinois. Such data are not currently available. Additionally, it was impossible to determine the strength of association between reported environmental factors and prevalence rates in dogs from our follow-up survey since no control group was surveyed, ie, practices that did not diagnose TBD were excluded from the follow-up survey.

Conclusions

There is little doubt that the number of human cases of TBDs in Illinois increased during the 2000–2009 decade. What is less certain is whether that trend reflected an actual increase in prevalence of these diseases or only indicated that more testing was being done, perhaps due to increased awareness among health-care providers and patients. Without access to the total number of TBD tests run in each year of the study period, we cannot be certain. A number of articles have suggested that an integrated public health surveillance system, one that would incorporate data from human health care, electronic medical records, veterinarians, climatologists, geospatial information systems, environmental and wildlife specialists, social scientists, syndromic systems, and social media, is critical as climate change affects our natural and built environment.[34–36] These data would include denominator data in the form of the total number of diagnostic tests run in addition to those tests that were positive for the trait of interest. Unless these data are available in a software platform that is readily useable, researchers will continue to be unable to tease out all of the many variables that determine the occurrence of disease and assess which risk factors are most important as determinants of disease risk. An open-source cyberenvironment for biosurveillance, which would accommodate these disparate sources of data, has been proposed.[37]

In addition to the need for an integrated surveillance system, communication between human health-care providers, veterinarians, and public health agencies regarding infectious diseases common to animals and humans has also been suggested as a need in the early diagnosis of shared and zoonotic diseases.[38] Indeed, authors have suggested that communication between veterinarians and physicians is largely absent.[39–41] Delay in the diagnosis of TBDs in humans has been reported to be a problem. Although the disease may not always be reportable in states where it is endemic, there is a case to be made for heightening awareness of TBD, since delay of diagnosis may be responsible for prolonged morbidity or even mortality.[42,43] Although an easy–to-use surveillance system that would accommodate data from health, social, weather, and environmental sources would be ideal, personal communication between health professionals is still key. In a previous paper, one of the authors proposed that local health departments serve as intermediaries for communication between veterinarians and human health-care providers.[38] If physicians and veterinarians are made aware of the prevalence and spatial distribution of these diseases within the communities that they serve, perhaps diagnostic testing in persons with compatible illness might occur sooner leading to improved health outcomes.

Author Contributions

Conceived and designed the experiments: JAH, NMD. Analyzed the data: JAH, NMD, MOR, WB. Wrote the first draft of the manuscript: JAH, NMD. Contributed to the writing of the manuscript: JAH, NMD, MOR. Agree with manuscript results and conclusions: JAH, NMD, MOR, WB. Jointly developed the structure and arguments for the paper: JAH, NMD, MOR, WB. Made critical revisions and approved final version: JAH, NMD, MOR, WB. All authors reviewed and approved the final manuscript.

REFERENCES

1. Chapman AS, Bakken JS, Folk SM, et al. Tickborne Rickettsial Diseases Working Group; CDC. Diagnosis and management of tick borne rickettsial diseases: Rocky Mountain spotted fever, ehrlichioses, and anaplasmosis – United States. *MMWR Recomm Rep*. 2006;55(RR04):1–27.
2. Centers for Disease Control and Protection. Summary of notifiable diseases – United States, 2008. *MMWR Recomm Rep*. 2010;57(54):1–94.
3. Little SE, Heise SR, Blagburn BL, Callister SM, Mead PS. Lyme borreliosis in dogs and humans in the USA. *Trends Parasitol*. 2010;26:213–8.
4. Wormser GP, Dattwyler RJ, Shapiro ED, et al. The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis*. 2006;43:1089–134.
5. Littman MP, Goldstein RE, Labato MA, Lappin MR, Moore GE. ACVIM small animal consensus statement on Lyme disease in dogs: diagnosis, treatment, and prevention. *J Vet Intern Med*. 2006;20:422–34.
6. Bowman D, Little SE, Lorentzen L, Shields J, Sullivan MP, Carlin EP. Prevalence and geographic distribution of *Dirofilaria immitis, Borrelia burgdorfei, Ehrlichia canis* and *Anaplasma phagocytophilum* in dogs in the United States: results of a national clinic-based serologic survey. *Vet Parasitol*. 2009;160:138–48.
7. Ettinger SJ, Feldman EC. *Textbook of Veterinary Internal Medicine*. 7th ed. Canada: WB Saunders; 2010:868–908.
8. Murphy GL, Ewing SA, Whitworth LC, Fox JC, Kocan AA. A molecular and serologic survey of *Ehrlichia canis, E. chaffeensis,* and *E. ewingii* in dogs and ticks from Oklahoma. *Vet Parasitol*. 1998;79:325–39.
9. Perez M, Bodor M, Zhang C, Xiong Q, Rikihisa Y. Human infection with *Ehrlichia canis* accompanied by clinical signs in Venezuela. *Ann N Y Acad Sci*. 2006; 1078:110–7.
10. Neer TM, Breitschwerdt EB, Greene RT, Lappin MR. Consensus statement on ehrlichial disease of small animals from the infectious disease study group of the ACVIM. *J Vet Intern Med*. 2002;16:309–15.
11. Chandrashekar R, Mainville CA, Beall MJ, et al. Performance of a commercially available in-clinic ELISA for the detection of antibodies against *Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Borrelia burgdorferi* and *Dirofilaria immitis* antigen in dogs. *Am J Vet Res*. 2010;71(12):1443–50.
12. Ismail N, Bloch KC, McBride JW. Human ehrlichiosis and anaplasmosis. *Clin Lab Med*. 2010;30:261–92.
13. Granick JL, Armstrong PJ, Bender JB. *Anaplasma phagocytophilum* infection in dogs: 34 cases (2000–2007). *J Am Vet Med Assoc*. 2009;234:1559–65.
14. Warner RD. Rocky mountain spotted fever. *J Am Vet Med Assoc*. 2002;221: 1413–7.
15. Paddock CD, Brenner O, Vaid C, et al. Short report: concurrent Rocky Mountain spotted fever in a dog and its owner. *Am J Trop Med Hyg*. 2002;66(2):197–9.
16. Elchos BM, Goddard J. Implications of presumptive fatal Rocky Mountain spotted fever in two dogs and their owner. *J Am Vet Med Assoc*. 2003;223:1450–2.
17. Kidd L, Hegarty B, Sexton D, Breitschwerdt E. Molecular characterization of *Rickettsia rickettsii* infecting dogs and people in North Carolina. *Ann N Y Acad Sci*. 2006;1078:400–9.
18. Parola P, Socolovschi C, Jeanjean L, et al. Warmer weather linked to tick attack and emergence of severe rickettsioses. *PLoS Negl Trop Dis*. 2008;2:338.
19. Gray JS, Dautel H, Estrada-Peña A, Kahl O, Lindgren E. Effects of climate change on ticks and tick-borne diseases in Europe. *Interdiscip Perspect Infect Dis*. 2009;2009:1–12.
20. Gatewood AG, Liebman KA, Vourc'h G, et al. Climate and tick seasonality are predictors of *Borrelia burgdorferi* genotype distribution. *Appl Environ Microbiol*. 2099;75:2476–83.
21. Suss J, Klaus C, Gerstengarbe FW, Werner PC. What makes ticks tick? Climate changes, ticks and tick-borne diseases. *J Travel Med*. 2008;15:39–45.
22. Climate Change in Illinois. Illinois State Water Survey. Available at http://www.isws.illinois.edu/atmos/statecli/Climate_change/iltrends.htm. Accessed June 17, 2011.
23. Climate Maps of the United States. National Climatic Data Center. Available at http://hurricane.ncdc.noaa.gov/climaps/temp0313.pdf. Accessed October 18, 2010.
24. Duncan AW, Correa MT, Levine JF, Breitschwerdt EB. The dog as a sentinel for human infection: incidence of *Borrelia burgdorferi* C6 antibodies in dogs from southeastern and Mid-Atlantic states. *Vector Borne Zoonotic Dis*. 2004;4:221–30.
25. Krupka I, Straubinger RK. Lyme borreliosis in dogs and cats: background, diagnosis, treatment and prevention of infections with *Borrelia burgdorferi* sensu stricto. *Vet Clin North Am Small Anim Pract*. 2010;40:1103–19.
26. Guerra MA, Walker ED, Kitron U. Canine surveillance system for Lyme borreliosis in Wisconsin and Illinois: geographic distribution and risk factor analysis. *Am J Trop Med Hyg*. 2001;65(5):546–52.
27. Reportable Communicable Disease Cases, 2000–2008. Illinois Department of Public Health. Available at http://www.idph.state.il.us/health/infect/communicablediseases00_09.htm. Accessed October 29, 2010.
28. Beall MJ, Chandrashekar R, Eberts MD, et al. Serological and molecular incidence of *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, and *Ehrlichia* species in dogs from Minnesota. *Vector Borne Zoonotic Dis*. 2008;8(4):455–64.
29. Tzipory N, Crawford PC, Levy JK. Incidence of *Dirofilaria immitis*, *Ehrlichia canis*, and *Borrelia burgdorferi* in pet dogs, racing greyhounds, and shelter dogs in Florida. *Vet Parasitol*. 2010;71:136–9.
30. Wright JC, Chambers M, Mullen GR, Swango LJ, D'Andrea GH, Boyce AJ. Seroincidence of *Borrelia burgdorferi* in dogs in Alabama, USA. *Prev Vet Med*. 1997;31:127–31.
31. Hadler JL. Disease Surveillance and Case Definitions in Tick-Borne Diseases in Critical Needs and Gaps in Understanding Prevention, Amelioration, and Resolution of Lyme and Other Tick-Borne Diseases: The Short-Term and Long-Term Outcomes. Washington, DC: Institute of Medicine; 2010:478.
32. US Census Bureau. *Quick Facts*. 2014. Available at http://quickfacts.census.gov/qfd/index.html#. Accessed May 16, 2011.
33. American Veterinary Medical Association. References – Estimate the Number of Pets. Available at http://www.avma.org/reference/marketstats/ownership.asp#formulas. Accessed June 18, 2011.
34. Pascal M, Viso AC, Medina S, Delmas MC, Beaudeau P. How can a climate change perspective be integrated into public health surveillance? *Public Health*. 2012;126:660–7.
35. Klompas M, McVetta J, Lazarus R. Integrating clinical practice and public health surveillance using electronic medical record systems. *Am J Prev Med*. 2012;42(6S2):S154–62.
36. Lenert L, Sundwall DN. Public health surveillance and meaningful use regulations: a crisis of opportunity. *Am J Public Health*. 2012;102(3):e1–7.
37. Edwards W, Vaid A, Brooks, I. INDICATOR: an open-source cyberenvironment for biosurveillance. *Defining Crisis Management* 3.0, Seattle, WA; 2010.
38. Herrmann JA, Kostiuk SL, Johnson YJ, Dworkin ME. Temporal and spatial distribution of blastomycosis cases among humans and dogs in Illinois (2001–2007). *J Am Vet Med Assoc*. 2011;239(3):335–43.
39. Kahn LH, Kaplan B, Steele JH. Confronting zoonoses through closer collaboration between medicine and veterinary medicine. *Vet Ital*. 2007;43(1):5–19.
40. von Matthiessen P, Sansone RA, Meier BP, Gaither GA, Shrader J. Zoonotic diseases and at-risk patients: a survey of veterinarians and physicians. *AIDS*. 2003;17(9):1404–6.
41. Grant S, Olsen CW. Preventing zoonotic diseases in immunocompromised persons: the role of physicians and veterinarians. *Emerg Infect Dis*. 1999;5:159–63.
42. O'Reilly M, Paddock C, Elchos B, Goddard J, Childs J, Currie M. Physician knowledge of the diagnosis and management of Rocky Mountain spotted fever – Mississippi, 2002. *Ann N Y Acad Sci*. 2003;990:295–301.
43. Helmick CG, Bernard KW, D'Angelo LJ. Rocky Mountain spotted fever: clinical, laboratory, and epidemiological features of 262 cases. *J Infect Dis*. 1984; 150:480–8.
44. CDC. Lyme Disease Diagnosis and Treatment. Available at http://wwwn.cdc.gov/NNDSS/script/casedef.aspx?CondYrID=752&DatePub=1/1/2011. Accessed July 16, 2013.
45. CDC. Ehrlichiosis and Anaplasmosis, 2008 Case Definition; 2008. Available at http://www.cdc.gov/osels/ph_surveillance/nndss/casedef/ehrlichiosis_2008.htm. Accessed October 31, 2010.
46. CDC. Rocky Mountain Spotted Fever, 2008 Case Definition; 2008. Available at http://www.cdc.gov/osels/ph_surveillance/nndss/casedef/rocky2008.htm. Accessed October 31, 2010.

Appendix 1. Case definitions for human TBDs

1. LD confirmed[44]

A case of EM with a known exposure (exposure is defined as having been (less than or equal to 30 days before onset of EM) in wooded, brushy, or grassy areas (ie, potential tick habitats) in a county in which LD is endemic. A history of tick bite is not required. OR

A case of EM with laboratory evidence of infection (as defined below) and without a known exposure OR

A case with at least one late manifestation that has laboratory evidence of infection.

Laboratory evidence of infection

Positive culture for *B. burgdorferi*, OR

Two-tier testing interpreted using established criteria1, where:

Positive immunoglobulin M (IgM) is sufficient only when ≤30 days from symptom onset

Positive IgG is sufficient at any point during illness

Single-tier IgG immunoblot seropositivity using established criteria.[1–4]

CSF antibody positive for *B. burgdorferi* by enzyme immunoassay or immunofluorescence assay (IFA), when the titer is higher than it was in serum

2. LD probable

Any other case of physician-diagnosed LD that has laboratory evidence of infection (as defined above).

3. Ehrlichiosis and anaplasmosis (previously human granulocytic ehrlichiosis) confirmed[45]

Defined as any reported fever and one or more of the following: headache, myalgia, anemia, leukopenia, thrombocytopenia, or any hepatic transaminase elevation and one of the following findings:

Demonstration of a fourfold change in antibody titer to *E. chaffeensis* (ehrlichiosis) or *A. phagocytophilum* (anaplasmosis) by IFA in paired samples; OR

Positive PCR assay; OR

Detection of *E. chaffeensis* or *A. phagocytophilum* by immunohistochemistry (IHC); OR

Culture of the organism.[35]

4. Ehrlichiosis and anaplasmosis (previously human granulocytic ehrlichiosis) probable

Defined as a clinically compatible case (meets clinical evidence criteria) that has supportive laboratory results. For ehrlichiosis/anaplasmosis – an undetermined case can only be classified as probable. This occurs when a case has compatible clinical criteria with laboratory evidence to support ehrlichia/anaplasma infection, but not with sufficient clarity to definitively place it in one of the categories previously described. This may include the identification of morulae in white cells by microscopic examination in the absence of other supportive laboratory results.

5. RMSF confirmed[46]

A confirmed case of RMSF is defined as any reported fever and one or more of the following: rash, eschar, headache, myalgia, anemia, thrombocytopenia, or any hepatic transaminase elevation and any of the following laboratory findings:

A fourfold change in IgG antibody titer to *R. rickettsii* antigen by IFA between paired serum specimens; OR

Detection of *R. rickettsii* by PCR; OR

Detection of *R. rickettsii* antigen by IHC; OR

Isolation of *R. rickettsii* in cell culture. (36)

6. RMSF probable

A probable case is one that is clinically compatible case (meets clinical evidence criteria) that has supportive laboratory results as defined as having serologic evidence of elevated IgG or IgM antibody reactive with *R. rickettsii* or other spotted fever group antigen by IFA, ELISA, dot-ELISA, or latex agglutination.

Appendix 2

1. LD is a difficult disease to confirm in dogs. A canine case of LD is defined as a positive ELISA test to *B. burgdorferi* (SNAP 3Dx or 4Dx) or Western blot and clinical signs of LD (fever, polyarthritis, lethargy, pain or nephritis).[3,5–7]
   a. **Note:** A positive test for *B. burgdorferi* on the SNAP 3Dx or 4Dx in the absence of any clinical signs will be considered an exposed canine case.[13]
2. A canine case of granulocytic anaplasmosis is defined as any one of the following:
   a. Detection of characteristic morulae in the cytoplasm of granulocytes on cytology; OR
   b. A positive ELISA, IFA, or PCR test to *A. phagocytophilum* and the presence of characteristic clinical pathology findings (thrombocytopenia) or clinical signs (fever, lethargy, anorexia, or lameness); OR
   c. A fourfold increase in IFA titers to *A. phagocytophilum* in acute and convalescent samples.[6,7,15,28]
   d. **Note:** A positive test for *A. phagocytophilum* by ELISA, IFA, or PCR in the absence of clinical signs or thrombocytopenia will be considered an exposed case.[13]
3. A canine case of monocytic ehrlichiosis (agent either *E. canis* or *E. chaffeensis*) is defined as any of the following:
   a. Detection of morulae in monocytes on cytology; OR
   b. A positive ELISA (SNAP 3Dx or 4Dx), IFA, or PCR test with clinical signs or clinical pathology characteristic of the disease (depression, lethargy, anorexia, nonregenerative anemia, thrombocytopenia, hyperglobulinemia); OR
   c. A fourfold increase in IFA titers in acute and convalescent samples.[6,7]
   d. **Note**: A positive ELISA test in the absence of any signs will be considered an exposed case.[13]
4. A canine case of RMSF is defined as one of the following:
   a. A greater than fourfold increase in IFA titers between acute and convalescent samples; OR
   b. An IFA titer of greater than or equal to 64 for IgM antibodies; OR
   c. A positive PCR test for *R. rickettsii*.[7,16]