ORIGINAL ARTICLE

White-Tailed Deer (*Odocoileus virginianus*) as a Potential Sentinel for Human Lyme Disease in Indiana

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Impacts

- Lyme disease (LD) is the most common tick-borne disease in the United States; however, the disease is often underreported because of diagnostic difficulties. Improvement of disease surveillance is essential for timely diagnosis and disease prevention.
- Here, we suggest a practical way of conducting an active surveillance for the disease, especially in areas where the disease is common, using ticks collected from hunted deer.
- This method will improve our ability to identify high-risk areas for LD infection and improve prevention and early diagnosis.

Keywords:
Borrelia burgdorferi; human Lyme disease; *Ixodes scapularis*; white-tailed deer; sentinel; SADIE; spatial association

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Summary

We assessed the potential of white-tailed deer (WTD) (*Odocoileus virginianus*) to be a sentinel for human cases of Lyme disease (LD) in Indiana using location data from a 3-year survey of approximately 3400 hunted deer with associated tick *Ixodes scapularis* and *Borrelia burgdorferi* (Bb) data. Data on human LD cases at the county level were obtained from the Indiana Department of Health. All data were assigned to county centroids to match the resolution of the LD data before creating optimized trend surfaces for LD incidence, hunted deer count, *Ixodes scapularis* and Bb prevalence. To determine whether LD was spatially associated with the areas of high densities of deer, deer with *Ixodes scapularis* and deer with ticks infected with Bb, we used spatial analysis with distance indices (SADIE). The SADIE analysis found significant spatial association between LD and the distribution of three organismal predictor variables, that is, WTD, *Ixodes* ticks and Bb. Lyme disease incident rate varied between 0.08 cases per 10 000 habitants (Johnson county) and 5.9 cases per 10 000 habitants (Warren county). In conclusion, WTD can be used as an accurate and cost-effective sentinel for human LD. This method will permit public health workers to identify potentially endemic areas independently of human case reports.

Introduction

Lyme disease (LD) is the most common vector-borne disease in the United States (Aguero-Rosenfeld et al., 2005). The causative organism, *Borrelia burgdorferi* (Bb), is transmitted by two tick vectors, *Ixodes scapularis*, distributed from the Midwest to the east coast, and *Ixodes pacificus* found on the west coast (Moody and Barthold, 1991). White-tailed deer (WTD; *Odocoileus virginianus*) is the principle host for the adult stage of *I. scapularis*, and consequently, deer count and *I. scapularis* populations have been found to be directly correlated (Ginsberg and Zhioua, 1999; Rand et al., 2004; Keefe et al., 2009). Like all other vector-borne zoonotic diseases, LD requires that the occurrence of the pathogen (Bb), its vector, one or more reservoir hosts, and the human victim be in relative proximity to one another. Tick densities and tick infection prevalence are two important ecological components of LD risk. A common assumption in studies of spatial patterns of LD incidence is that LD is contracted

peridomestically, and this is supported by the finding that patients with LD in endemic areas often have infected ticks in their yards or nearby forest fragments (Falco and Fish, 1988; Maupin et al., 1991; Connaly et al., 2006). Nevertheless, in most cases, testing this assumption is difficult because it requires rarely documented additional information regarding patients' behaviour and travel around the time of potential contact with the vector tick (Connaly et al., 2006).

On the other hand, an unknown but potentially significant proportion of cases may involve contracting LD away from the home. In areas of the United States with very sparse or non-existent tick populations, locally reported cases most likely represent exposure during travel to endemic areas. Of particular interest are intermediate areas such as the Midwest, especially Illinois and Indiana where the presence of the pathogen has been reported but not on the same scale as in the North-east (Pinger et al., 1996).

Free-ranging wildlife has been suggested as a necessary component in the development of practical strategies for conducting surveillance to detect, monitor and predict zoonotic diseases. Deer have been proposed as a sentinel animal for a variety of zoonotic diseases including LD. Magnarelli et al. (1993, 1995) looked for both *I. scapularis* and Bb in hunted deer in Connecticut but did not look for any association with human LD. Gallivan et al. (1998) and Magnarelli et al. (2010) suggested WTD as a sentinel for LD following a serologic survey in Ontario, Canada, and Connecticut, USA, respectively. Only three studies have assessed the association between the cases of human LD and *I. scapularis* distribution. None have assessed the association of human LD and *I. scapularis* with Bb. Schulze et al. (1984) reported an association between human cases of LD and the distribution of *I. scapularis* collected from 567 WTD within New Jersey, USA. Amerasinghe et al. (1992) used county-based cases of human LD in Maryland, USA, and found a high correlation with *I. scapularis* collected from 538 hunted deer. Similarly, Daniels et al. (1993) found a similar correlation for *I. scapularis* in endemic and non-endemic areas in Connecticut. Nevertheless, no study has reported on the association between Bb detected in *I. scapularis* collected from deer and human LD. Furthermore, dogs have been extensively studied as suitable sentinels for human LD (Lindenmayer et al., 1991; Daniels et al., 1993; Olson et al., 2000; Duncan et al., 2005; Hamer et al., 2009). A few of these studies, however, assessed the association between human and dog LD (Lindenmayer et al., 1991; Daniels et al., 1993; Guerra et al., 2001) using serum samples. However, because of increasing vaccination against LD among dogs, especially in high-risk areas (Guerra et al., 2001), this approach can bias the actual association.

The objective of this study was to asses whether WTD can serve as an accurate sentinel for the presence of human LD by taking advantage of a comprehensive active surveillance programme that yielded a detailed distribution of infected and non-infected *I. scapularis* (Keefe et al., 2009; Raizman et al., 2010). This information is needed to help in the development of an accurate and economically sustainable surveillance programme for human LD in Indiana and other areas. We hypothesized that the distribution of WTD infested with *I. scapularis* and Bb can serve as an accurate sentinel for LD cases in Indiana. We therefore tested predictions that the cases of human LD would be spatially associated with deer count, count of *Ixodes*-infested deer and prevalence of Bb.

Material and Methods

Tick sampling

Data on the occurrence of deer, *Ixodes* ticks and *B. burgdorferi* were obtained from an extensive survey of deer brought to hunting check-in stations across the state during the first weekend of rifle hunting season in 2005, 2006 and 2007. These data were collected in 82 of Indiana's 92 counties (89%) as detailed elsewhere (Keefe et al., 2009; Raizman et al., 2010). We conducted tick collections from hunter-harvested WTD reported at designated checkpoint stations in Indiana on the opening weekend (2 days) of firearm season (mid-November) in 2005, 2006 and 2007. A single station was visited per county selected, per day. Volunteers were grouped into pairs for both sampling days. The consistent November sampling date minimized weather from greatly affecting tick numbers from year to year. One additional sampling each year was performed on the last Monday and Tuesday of November in Pulaski County during Tippecanoe River State Park's closed hunt. Hunters were asked to indicate the location of deer collection using a road atlas map at a scale of 1 : 156 000. Deer were checked for ticks over a consistent part and proportion of the carcass. Ticks discovered were removed from the carcass for molecular analysis.

Molecular detection of *B. burgdorferi*

Polymerase chain reaction (PCR) testing was performed at the laboratory of Dr Moro at the Kansas State University Department of Veterinary Pathobiology. Ticks were processed in pools of four ticks or less where all ticks in the same pool were from the same sampled deer. All pools were subjected to an initial wash in a 10% bleach solution for 3 min, rinsed in sterile water and cut with a sterile scalpel blade. DNA was purified using the QIAamp DNA micro kit (Qiagen, Valencia, CA, USA). Primers specific for the flagellin gene of Bb (forward primer:

5′-TTA ATC GAG CTT CTG ATG ATG CTG C-3′ and reverse primer 5′ ATT TCG TCT GTA AGT TGC TCT ATT TCA A-3′) were used to detect presence in pooled tick DNA samples by PCR (Germer et al., 1999). PCR was performed using HotStarTaq master mix (Qiagen) on a Master Cycler (Eppendorf, Westbury, NY, USA) as follows: initial incubation at 95°C for 15 min, 94°C for 90 s, 54°C for 90 s and 72°C for 30 s. A total of 35 cycles were applied, followed by incubation at 72°C for 7 min. PCR products were separated by electrophoresis on a 2% agarose gel and visualized using ethidium bromide. *Borrelia burgdorferi* strain N40 was used as a positive control for PCR. As a control for DNA extraction and purification, and to rule out endogenous PCR inhibitors in each sample, PCR amplification was conducted on a 1485-bp gene sequence of bacterial 16S rRNA as previously described (Barbieri et al., 2001). All samples positive for Bb based on the flagellin gene were then amplified with a secondary marker for Bb sensu stricto (s.s.) using PCR primers specific for the *Borrelia* ospA gene (Norris et al., 1997). OspA PCR products were cloned into the pGEM-T cloning vector (Promega, Madison, WI) and sequenced. Cloned fragments were analysed by BLAST against the National Center for Biotechnology Information NR database at GenBank. Fragments having 99% or greater similarity to the ospA gene at the nucleotide level were considered confirmation of Bb s.s.

Statistical analysis

To examine the relationship between reported cases of LD in humans and the counts of deer, *Ixodes scapularis* and *I. scapularis* carrying Bb, we correlated their extrapolated values at Indiana county centroids. This allowed us to summarize the various predictor variables (i.e. number of hunted deer, number of deer with *I. scapularis* and number of deer with I. scapularis with *I. scapularis*) to match the finest resolution available for the disease occurrence data. The number of reported cases of LD in the period 2004–2008 for each county in Indiana was obtained through the Indiana Department of Health (IDH). These data were assigned to county centroids in ArcGIS (ver. 9.3; ESRI, Redlands, USA). Analysis using individual point data for human LD cases was not available to match the WTD, *I. scapularis* and Bb data because LD data were available at the county level only. Approximately 3400 point locations of hunted deer resulted with associated tick and Bb data. To generate for each county, a predicted number of deer, deer infested with ticks and deer infested with Bb-infested ticks, we first summed the individual occurrences from the original point data within each county. We imported the point locations of county centroids and associated human LD incidence data, as well as WTD and associated *I. scapularis* and Bb data, into GRASS GIS (GRASS Development Team, 2010). Additionally, we took county human population data from the 2000 census and assigned these to the centroids of Indiana's 92 counties. We calculated LD incidence as the number of cases for each 10 000 human inhabitants per county. We then used the v.surf.rst package (Neteler and Mitasova, 2004) in GRASS to create a smooth surface through the centroids of the 92 counties for the three predictor variables and the LD incidence data. The v.surf.rst package creates a raster layer (i.e. a grid of square cells with x and y coordinates and a cell value) that represents a regularized spline surface through the points (e.g. county centroids). A regularized spline surface is a smooth surface created using interpolation functions and leads to realistic slopes that are close to orthogonal to the contour lines (height isolines) (Mitasova and Hofierka, 1993). The software user is able to control the unitless tension and smoothing parameters that influence the character of the surface and how closely it represents the centroids values at the points (best visualized as the surface height) (Neteler and Mitasova, 2004). To find the best combination of tension and smoothing parameters, with tension between 10 and 100 in increments of 5 and smoothing between 0 and 100 in increments of 2, we needed to examine the fit of 969 possible surfaces. We therefore used a Linux BASH script to run parameter sweeps across all tension and smoothing combinations. For the three predictor variables, we used square-root transformed data to avoid isolated high values from having undue influence on the surface and thereby forcing the smoothing values to a more malleable surface, without the need to omit outliers. For each of these candidate surfaces, a leave-one-out jackknife procedure (Miller, 1974) was used to compare the values predicted to those assigned to the centroids. This procedure sequentially omitted one of the county centroids and estimated the value at this point based on the surface generated with all other points. This process was repeated for all points, and using the R statistical platform (R Development Core Team, 2009), we calculated a sum of squared errors (SSE) value for each candidate surface from the jackknife procedure. We compared the 969 possible surfaces and selected the one with the lowest SSE as the best surface for that organism. The values predicted at each county centroid, including those for the 10 counties not surveyed, were then extracted from the best fitting surface.

To determine whether the predictor variables were spatially associated with LD occurrence, we used spatial analysis with distance indices analysis (SADIE; Perry et al., 1996) on the predicted county centroid data separately for each variable from the surface analysis above. SADIE

analysis identifies statistically significant hot and cold spots (i.e. regions with significantly higher or lower values than expected) while correcting for spatial autocorrelation with Dutilleul's adjustment (Dutilleul, 1993). Spatial autocorrelation can result in a lowering of the appropriate degrees of freedom in a statistical test because points are not completely independent from neighbouring points. The association test within SADIE then uses the maps of local spatial aggregation statistics from each variable to determine whether these second-order values (i.e. aggregation statistics) are spatially correlated between LD and one of the three predictor organisms. The calculated X statistic is analogous to a correlation coefficient but is calculated on the aggregation values. It is the sum of the products of the cluster indices for the two variables at each point. It therefore determines whether the areas of higher or lower aggregation than expected by chance are located in the same areas for the different variables. Statistical significance is tested with a randomization procedure (Perry et al., 1996), and we created the null distribution from 9999 randomizations. We used a one-tailed test with a significance level of $P < 0.05$ as we predicted positive relationships between cases reported and all other variables.

Results

Between 2005 and 2007, a total of 3412 deer were searched for ticks in 82 (89%) of Indiana's 92 counties (Keefe et al., 2009; Raizman et al., 2010). The median number of deer checked in at each station each year was 37. A total of 4136 *Ixodes scapularis* were collected during this period. A total of 1962, 646 and 1528 were collected each year, respectively. *Ixodes scapularis* ticks were collected on 13.6%, 15.8% and 25.5% of the sampled deer in these years, respectively, and in 63% of the 82 sampled counties. Bb was identified in 11 counties (12%). Fifty-five counties (60%) had reported cases of LD during 2004–2008. From 16 of these 55 counties (29%), *I. scapularis* was not recovered at all and from another 6 (11%) counties sampling did not take place. The number of cases in counties with LD reports varied from one to twenty, and the mean number of cases in these counties with reports was 3.4 ± 4.5. Incidence rate within counties varied between 0.08 (0.11 adjusted for best surface) for 10 000 habitants in Johnson County and 5.9 (1.55 adjusted) in Warren county, with a mean and std of 0.6 (adj. 0.4) and 0.81 (adj. 0.31), respectively.

The best fit surfaces selected from the 969 surfaces for each variable are shown in Fig. 1. High deer numbers were located in two large foci in the northern and southern parts of the state (Fig. 1a), whereas *I. scapularis* and Bb foci match the deer counts in the northern part of the state (Fig. 1b,c). In Indiana, the spatial trend in human density (Fig. 1d) is driven by highly populated areas like Indianapolis and Gary. The number of LD cases is much higher close to highly populated urban areas such as the

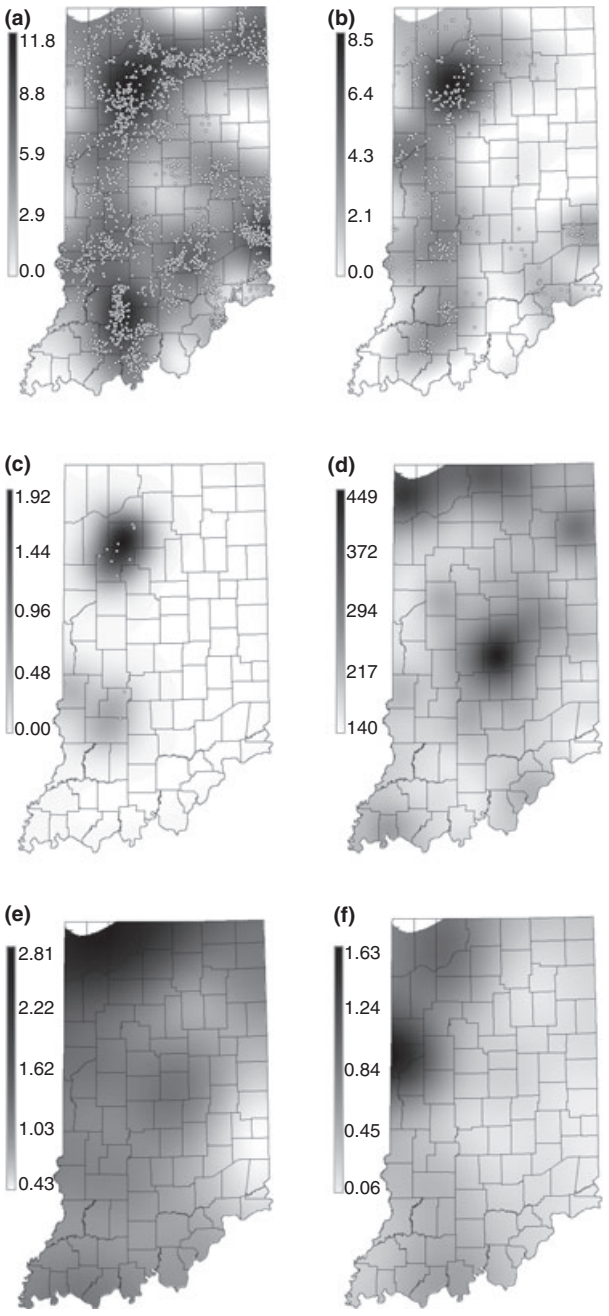

Fig. 1. Optimized surfaces predicting: (a) deer counts, (b) *Ixodes* tick counts, (c) *B. burgdorferi* prevalence, (d) human density, (e) Lyme disease (LD) cases and (f) LD incidence. Points in a–c show survey locations in Indiana (2005–2007) where relevant organism was detected. Scale bars show square-root value scale used to fit surfaces through county centroids.

Lake Michigan area and Chicago, Illinois (Fig. 1e). Human LD incidence (Fig. 1f) is driven by the low population as a denominator relative to the high number of cases. The SADIE analysis found significant correlations between the incidence of human LD and all three of the predictor variables, that is, deer, deer infested with *I. scapularis* and *I. scapularis* ticks infected with Bb (Table 1).

Discussion

Our results indicate a statistically significant correlation between human LD incidence and the distribution of deer and *I. scapularis*-infested deer whether infected or not infected with Bb. Hence, it is plausible to state that deer can serve as an accurate sentinel for human LD. This confirms and reinforces our results from a previous report about the use of hunted deer to describe the distribution of Bb (Keefe et al., 2009). Further, this study emphasizes the concept that ecological data on vector-borne zoonosis are essential in understanding and predicting human risk. Indiana appears to be an emerging area for LD (Pinger, 2008), and this information will have local and regional importance.

To the best of our knowledge, this is the first study in two decades to assess the use of WTD as a sentinel for human LD by matching LD human cases with a precise distribution of Bb-infected and non-infected adult-stage *I. scapularis* at as large an extent as the state of Indiana. Further, it is the first to report a significant association between the presence of Bb and human LD incidence. The association with *I. scapularis* distribution was previously evaluated in only three studies (Schulze et al., 1984; Amerasinghe et al., 1992; Daniels et al., 1993). In addition to a complex spatial analysis, which was not available two decades ago, our study used a significantly larger sample size. All other survey studies using ticks collected from deer did not assess the correlation between the distribution of either the tick or Bb with human cases of LD. One strength of the present study is the precise location information of both infected and non-infected tick distributions collected from harvested deer during 3 years of surveys (Keefe et al., 2009). Nevertheless, because we used deer as a sentinel for the adult stage of *I. scapularis* distribution and deer are known to move large distances, our results do not necessarily reflect the nymph distribution, which is known to be the main source of human Bb infections.

Table 1. Correlation results from spatial analysis with distance indices analysis of hunted deer, *Ixodes scapularis*, *Borrelia burgdorferi* and human incidence of Lyme disease (LD) at the county level in Indiana

Test	n	Adj. n	*X* stat.	Adj. *P*
LD – deer	92	33.7	0.3457	0.0216[a]
LD – *Ixodes*	92	28.8	0.6255	0.0002[a]
LD – *B. burgdorferi*	92	23.5	0.7041	<0.0001[a]

n, sample size; adj. n, adjusted sample size; *X* stat., test statistic of spatial association; adj. *P*, *P* value after Dutilleul's adjustment
[a]Significant *P* value.

In our analysis, we used incidence as a measure of risk, where there is more weight given to population than number of cases. Incidence rates for sparsely populated counties are driven more by the small denominators (population) than by the numerator (number of cases), compared to highly populated areas. As a result, the incidence suggests that a high-risk area exists in a sparsely populated county in west-central Indiana (Fig. 1f). This coincides with our findings about the distribution of Bb.

The correlation between WTD, the tick and Bb and human LD suggests that within Indiana, people are likely become infected with Bb beyond the peridomestic area, as was previously suggested (Falco and Fish, 1988; Maupin et al., 1991; Connaly et al., 2006), though possibly still within the county, at areas such as state parks and forests during different recreational activities. While most LD cases were diagnosed and reported in counties with large urban centres, most of the *I. scapularis* and Bb were found outside these areas (Fig. 1). Hence, it also possible that LD is acquired outside the urban areas by their residents but is diagnosed once the people return to the urban areas. The higher environmental risk of being infected by Bb outside the urban areas, as our Bb distribution suggests, is also supported by the significantly higher incidence rate in sparsely populated counties compared to large urban areas.

It is possible that the distribution of Bb-infected ticks is larger than described in this study, certainly among the nymph stages of *I. scapularis*. Lacombe et al. (1993) compared the rates of infection with Bb in adult *I. scapularis* collected from deer at Maine, USA, with those collected from vegetation at the same sites using polyclonal direct fluorescent antibody test and reported higher infection rates (47%) among ticks from vegetation compared to ticks from deer (13%). This discrepancy, however, is probably due to the limited test sensitivity. Deer blood has been shown to have borreliacidal properties (Nelson et al., 2000). Hence, it is possible that blood-fed adult ticks have less detectable Bb than host-seeking adult ticks. Additionally, these borreliacidal prosperities can cause inhibition of the PCR assay (Schwartz et al., 1997) leading to underestimation of the prevalence. In Indiana, as in other states, LD cases are reported on the basis of the passive surveillance case definition and therefore LD is probably underreported (Meek et al., 1996). It is also likely that LD is not being diagnosed or reported because

of diagnostic difficulties, as in the case of *erythema migrans* in patients with darker skin (Fix et al., 2000). Counties with a longer history of LD are likely to have greater awareness among health care providers. These factors could influence the spatial correlation in some areas where ticks are abundant.

Because of strict confidentiality legislation, IDH could provide LD cases only on the county level. This restricted our ability to assess the correlation on a finer resolution, such as at the town or township level.

In conclusion, our analysis shows that WTD can serve as an accurate sentinel for human cases of LD. This method will permit public health workers to identify endemic and potentially endemic areas independently of human case reports. Further, in addition to the peridomestic risk of contracting LD, other local habitats favouring the presence of the vector tick, such as deciduous forest (Raizman et al., 2010), are a significant risk factor for local habitants to contract the disease.

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