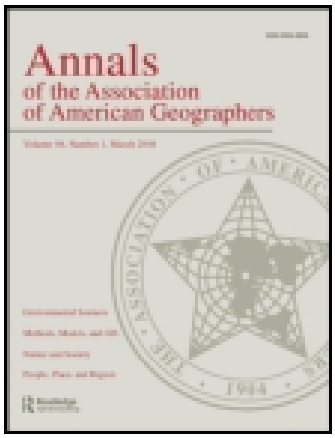

Annals of the Association of American Geographers
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/raag20

Spatial and Temporal Patterns of Anthrax in White-Tailed Deer, Odocoileus virginianus, and Hematophagous Flies in West Texas during the Summertime Anthrax Risk Period

Jason K. Blackburn[a], Ted L. Hadfield[b], Andrew J. Curtis[c] & Martin E. Hugh-Jones[d]

[a] Spatial Epidemiology & Ecology Research Laboratory, Department of Geography, University of Florida

[b] MRI Global

[c] GIS, Health & Hazards Lab, Department of Geography, Kent State University

[d] Department of Environmental Sciences, School of the Coast and Environment, Louisiana State University

Published online: 11 Jun 2014.

To cite this article: Jason K. Blackburn, Ted L. Hadfield, Andrew J. Curtis & Martin E. Hugh-Jones (2014) Spatial and Temporal Patterns of Anthrax in White-Tailed Deer, Odocoileus virginianus, and Hematophagous Flies in West Texas during the Summertime Anthrax Risk Period, Annals of the Association of American Geographers, 104:5, 939-958, DOI: 10.1080/00045608.2014.914834

To link to this article: http://dx.doi.org/10.1080/00045608.2014.914834

Spatial and Temporal Patterns of Anthrax in White-Tailed Deer, *Odocoileus virginianus*, and Hematophagous Flies in West Texas during the Summertime Anthrax Risk Period

Jason K. Blackburn,* Ted L. Hadfield,† Andrew J. Curtis,‡ and Martin E. Hugh-Jones§

*Spatial Epidemiology & Ecology Research Laboratory, Department of Geography, University of Florida
†MRI Global
‡GIS, Health & Hazards Lab, Department of Geography, Kent State University
§Department of Environmental Sciences, School of the Coast and Environment, Louisiana State University

White-tailed deer, *Odocoileus virginianus*, anthrax epizootics have been frequently documented in Texas over the last two decades. Once outbreaks begin, there is evidence for the potential role of hematophagous flies as vectors for the disease. Hypotheses on the role of biting flies in the transmission of anthrax date back more than a century. Both laboratory experiments and field studies have provided evidence of a biting fly transmission pathway. In particular, several studies have implicated biting flies during severe wildlife outbreaks in North America. Despite these implications, there is a lack of spatial analysis relating flies and anthrax. Here we report on the spatial patterns of anthrax in white-tailed deer on a well-studied ranch with a documented anthrax history. These patterns were evaluated against the spatiotemporal patterns of biting flies during the anthrax risk period. Unbaited fly traps were used to collect flies across the study ranch from June through August 2005. Kernel density analysis confirmed biting fly hotspots concentrated in the areas with highest densities of deer carcasses. The average nearest neighbor index confirmed that deer carcasses were spatially clustered and density estimates suggest that these are in proximity to areas supporting high fly populations. Dual kernel density analysis of carcasses and deer population identified a large dry riverine habitat as a high anthrax risk. Fly catch rates across the period identified a similar pattern to the anthrax risk surface. The high overlap between areas of sustained high fly catch rates and anthrax cases does suggest a relationship warranting future research. *Key Words: anthrax, disease ecology, hematophagous flies, spatial epidemiology, white-tailed deer.*

过去二十年来, 德州经常记录到学名为 Odocoileus virginianus 的白尾鹿以及动物炭疽流行病。有证据显示, 疫情一旦爆发, 噬血苍蝇在疾病传播上扮演着潜在角色。有关叮咬苍蝇在炭疽病传播中的角色之假说, 可追溯至一个世纪以上。实验室的实验与田野研究, 皆提供了叮咬苍蝇的传染途径之证据。在北美几次疫情严重的野生动物流行病爆发中, 部分研究特别指涉了叮咬苍蝇的角色。儘管有着上述指涉, 但目前仍然缺乏有关苍蝇和炭疽病的空间分析。我们于此报导在一个具有炭疽病历史纪录并受到详尽研究的牧场中, 白尾鹿群中的炭疽病空间模式。这些模式, 将对照炭疽病危险时期的叮咬苍蝇之时空模式进行评估。我们在 2005 年六月至八月间, 使用未设圈套的苍蝇诱捕器来蒐集研究牧场中的苍蝇。核密度分析証实, 叮咬苍蝇的热点集中于鹿群尸体密度最高的地区。平均最邻近指标证实, 鹿群尸体具有空间集中性, 而密度评估则指出, 这些尸体集中处邻近支持大量苍蝇群体的地区。尸体与鹿群的双核密度分析, 指出大量的近河畔乾燥栖地具有高度的炭疽病风险。在此期间的苍蝇捕获率, 与炭疽病风险的表面模式相似。持续具有高度苍蝇捕获率的区域与炭疽病案例之间的高度重叠, 则指出未来研究应关注的关係。 关键词: 炭疽病, 疾病生态学, 噬血苍蝇, 空间流行病学, 白尾鹿。

La epizoótica ántrax del ciervo cola blanca (*Odocoileus virginianus*) ha sido frecuentemente documentada en Texas durante las últimas dos décadas. Tan pronto como empieza un brote, se alude a la evidencia del papel potencial que pueden asumir las moscas hematófagas como vectores de la enfermedad. Las hipótesis sobre el papel de las moscas picadoras en la trasmisión del ántrax se remontan a más de un siglo. Tanto experimentos de laboratorio como los estudios de campo han aportado evidencia sobre una ruta de trasmisión de la enfermedad por picaduras de moscas. Varios estudios en particular han implicado estas picaduras durante brotes severos que afectan la vida silvestre en América del Norte. Pero a pesar de tales implicaciones, se nota la falta de análisis espaciales que relacionen a las moscas con el ántrax. En el presente trabajo informamos sobre los patrones espaciales del ántrax

Annals of the Association of American Geographers, 104(5) 2014, pp. 939–958
Initial submission, October 2012; revised submission, July 2013; final acceptance, September 2013
Published by Taylor & Francis, LLC.

en el ciervo cola blanca detectados en un rancho que fue muy bien estudiado con una historia documentada de la enfermedad. Los patrones observados se evaluaron frente a los patrones espacio-temporales de estas moscas durante el período de riesgo de ántrax. Se utilizaron trampas sin cebo para atrapar moscas en el territorio del rancho bajo estudio entre junio y agosto de 2005. El análisis de densidad kernel confirmó la existencia de zonas críticas concentradas en las áreas que registraron las máximas densidades de restos de ciervos. El promedio del índice de vecino más cercano confirmó que los restos de los ciervos muertos estaban espacialmente aglomerados y los cálculos de densidad sugieren que éstos se hallan en la proximidad de áreas que están infestadas con altas poblaciones de moscas. El análisis de densidad kernel dual de los restos y de la población de ciervos identificó un hábitat fluvial seco grande como área con alto riesgo de ántrax. Las tasas de captura de moscas durante el período de estudio identificaron un patrón similar para la superficie de riesgo de ántrax. El alto traslape registrado entre las áreas de tasas sostenidas de alta captura de moscas y los casos de ántrax en verdad sugieren una relación que amerita más investigación. *Palabras clave: ántrax, ecología de enfermedades, moscas hematófagas, epidemiología espacial, ciervo cola blanca.*

Anthrax remains a problem for both wildlife and livestock in parts of North America. In particular, white-tailed deer, *Odocoileus virginianus*, epizootics have been frequently documented in Texas over the last decade (Hugh-Jones and De Vos 2002; Blackburn 2006; Hugh-Jones and Blackburn 2009). Recently, researchers suggested that anthrax is an underdiagnosed and underappreciated disease requiring greater attention (Fasanella et al. 2010), including the need for wildlife surveillance (Blackburn et al. 2007; Fasanella et al. 2007). Despite being a disease of antiquity, the ecology of *Bacillus anthracis*, the etiological agent of anthrax, is still relatively poorly understood (Smith et al. 2000; Hugh-Jones and Blackburn 2009). *Bacillus anthracis* is a spore-forming, soil-borne bacterium (Van Ness 1971; Smith et al. 2000). Most recently, Alexander et al. (2012) defined naturally occurring anthrax as an obligate spillover disease, with the pathogen linked directly to the environmental conditions associated with maintenance or persistence. The dominant transmission hypothesis is *B. anthracis* reproduces by infecting vertebrate hosts (primarily large vertebrate herbivores), through ingestion of spores during browsing or grazing, including exposure from soil ingestion (Smith et al. 2000; Turnbull et al. 2008). Spores then germinate in the host and replicate, eventually resulting in death of the host animal. When blood or body fluids containing bacilli are spilled into the environment or exposed to oxygen, the vegetative cells sporulate and eventually contaminate the soil. Evidence suggests that there might also be potential for spore maintenance in the rhizosphere of certain grasses (Saile and Koehler 2006), which might ultimately play a role in persistence while promoting infection of grazing herbivores. Inhalation of spores from the environment has limited evidence but cannot be ruled out (Turnbull et al. 2008). Once outbreaks begin, there is evidence for the potential role of both necrophagous (Blackburn et al. 2010) and biting insects, primarily hematophagous flies, as potential vectors for the disease (Gates, Elkin, and Dragon 1995). A series of studies confirmed the successful isolation and transmission of *B. anthracis* by biting flies under both field and laboratory conditions. Ganeva (2004) summarized a number of fly studies specific to anthrax and documented that at least twenty-one species representing five genera from the Tabanidae family could mechanically transmit anthrax bacilli on body parts such as the legs and mouth parts.

Hypotheses on the role of biting flies in the transmission of anthrax date back to the early part of the last century. Morris (1918) confirmed that lethal concentrations of bacilli could be transmitted to guinea pigs by flies of the genus *Tabanus*. That study indicated that transmission was most successful within the first four hours following a blood meal. Turell and Knudson (1987) found similar results for *Stomoxys calcitrans*, another species of biting fly, and two species of mosquitoes under experimental conditions. Their study confirmed transmission of disease with flies allowed to feed first on infected guinea pigs and then disease-free guinea pigs. It also indicated that transmission was most successful within the first four hours of a blood meal but did not conclude that transmission potential was limited to the first several hours after a blood meal.

Although the work of Turell and Knudson (1987) was limited to laboratory experiments, there is also evidence for transmission of bacilli during outbreaks. Mohiyudeen and Krishna Rao (1958) isolated *B. anthracis* in blood smears from cutaneous lesions on affected bovines during an outbreak in India. Additionally, biting flies collected on those anthrax-positive cows tested positive for *B. anthracis*. They reported that the outbreak spread rapidly and covered a large geographic distribution by its completion. Other field studies have implicated biting flies in wildlife outbreaks, although no active sampling was completed to confirm

the presence of the bacteria in flies. Broughton (1992) and Gates, Elkin, and Dragon (1995) anecdotally documented the association of high biting fly numbers and high numbers of anthrax deaths in wood bison, *Bison bison athabascae*, in northern Canada. A major white-tailed deer epizootic in Arkansas was partially attributed to high biting fly densities (Kellogg, Prestwood, and Noble 1970). Additionally, Olsuf'ev and Lelep (1935) associated high anthrax case numbers in livestock with high biting fly numbers. The Olsuf'ev study showed the peak in anthrax cases lagging behind the peak of fly numbers by ten days. Hugh-Jones and De Vos (2002) hypothesized that biting flies could increase the spatial footprint of an outbreak. To date, however, no studies have linked such spatial patterns with positive confirmation of the role of biting flies.

There is further historical evidence of biting fly involvement in anthrax. Elliott (1955) reported isolation of *B. anthracis* from two horseflies in southeast Louisiana during a large epizootic in 1954. The first fly was collected from a dying horse near Venice and the second was collected from a cow near New Orleans. Bacteria isolated from both produced especially pathogenic infections in mice. Abdulla et al. (1982) suggested that biting flies were the most likely source of fatal infection for a captive jaguar at a zoo in Kerala, India, in 1981. There was no apparent link between the case and water or meat, as both were regularly tested and widely distributed to other carnivores in the zoo without incident. Bales and others (2002) reported a human cutaneous anthrax case believed to be the result of a fly bite during a cattle burning in Inyo County, California, in 1968.

Despite a number of studies implicating flies in outbreaks, there is a lack of spatial analysis relating them to anthrax, as has been noted in other arthropod vector studies (Jeffery et al. 2002). The growing field of spatial analyses and geospatial statistics lends itself to spatially explicit analyses in livestock and wildlife epizootiological studies, however (Ward and Carpenter 2000). For example, Jeffery et al. (2002) employed kriging and a measure of local spatial autocorrelation to define the distribution of mosquito vectors for Ross River and Barmah Forest viruses in Australia. Similarly, Sciarretta et al. (2005) employed geostatistical methods to model the seasonal variation of tsetse flies (*Glossinia spp.*) in Ethiopia. Such studies are important for understanding the potential role of biting flies, as vectors and hosts must overlap in environments that support the pathogen for successful transmission to occur.

This study addresses the need to evaluate the relationship between biting flies and wildlife anthrax. We first report on the spatial and temporal patterns of anthrax in white-tailed deer on our study ranch in the enzootic anthrax region of west Texas. We then employed kernel density–based hotspot and spatial prevalence mapping of deer mortality events from an anthrax epizootic in 2005. Next we employed spatial clustering statistics in the analysis of biting fly collections from the 2005 anthrax season at the study site. The goal of this study was to test whether biting flies exhibit a uniform distribution across the study area or cluster in areas associated with high numbers of anthrax-positive deer carcasses during the risk period and to evaluate the ecological conditions that support high fly densities.

Materials and Methods

This study employed anthrax surveillance data from a deer herd and biting fly collections that were conducted on a wildlife ranch located approximately 80 km north of Del Rio, Val Verde County, Texas (Figure 1). Ecologically the ranch is situated on the Edwards Plateau ecoregion based on the Level III classification scheme (Yanli et al. 2009). The ranch encompasses ~7,406 ha and is managed exclusively for white-tailed deer and game birds. Well-documented anthrax outbreaks occurred on the ranch in recent years (see Table 1 and Figure 1), and active surveillance for dead deer was conducted by ranch staff and researchers annually during the anthrax season from 2001 to present. In Texas, the anthrax risk period is primarily from late spring thru early fall (mid-May–early October; Hugh-Jones and Blackburn 2009). To date, anthrax cases have only been found in the central and eastern areas of the ranch, despite ranch-wide surveillance. Our work in the larger area surrounding the ranch from 2002 to present identified this area as an enzootic zone with cases confirmed nearly every year on this and ranches across the zone (Figure 1). The exact boundary of the enzootic zone remains fuzzy at best. To define the boundary, we used the county boundaries of those counties with reported cases since 2000. To illustrate the fuzziness of the zone, we used a semitransparent boundary.

Anthrax Mapping

Deer carcass locations on the ranch were recorded with Global Positioning System (GPS) units and mapped from 2003 to 2010. Carcasses were located by ground searches. In addition to carcasses directly sighted, anthrax spotters relied heavily on turkey

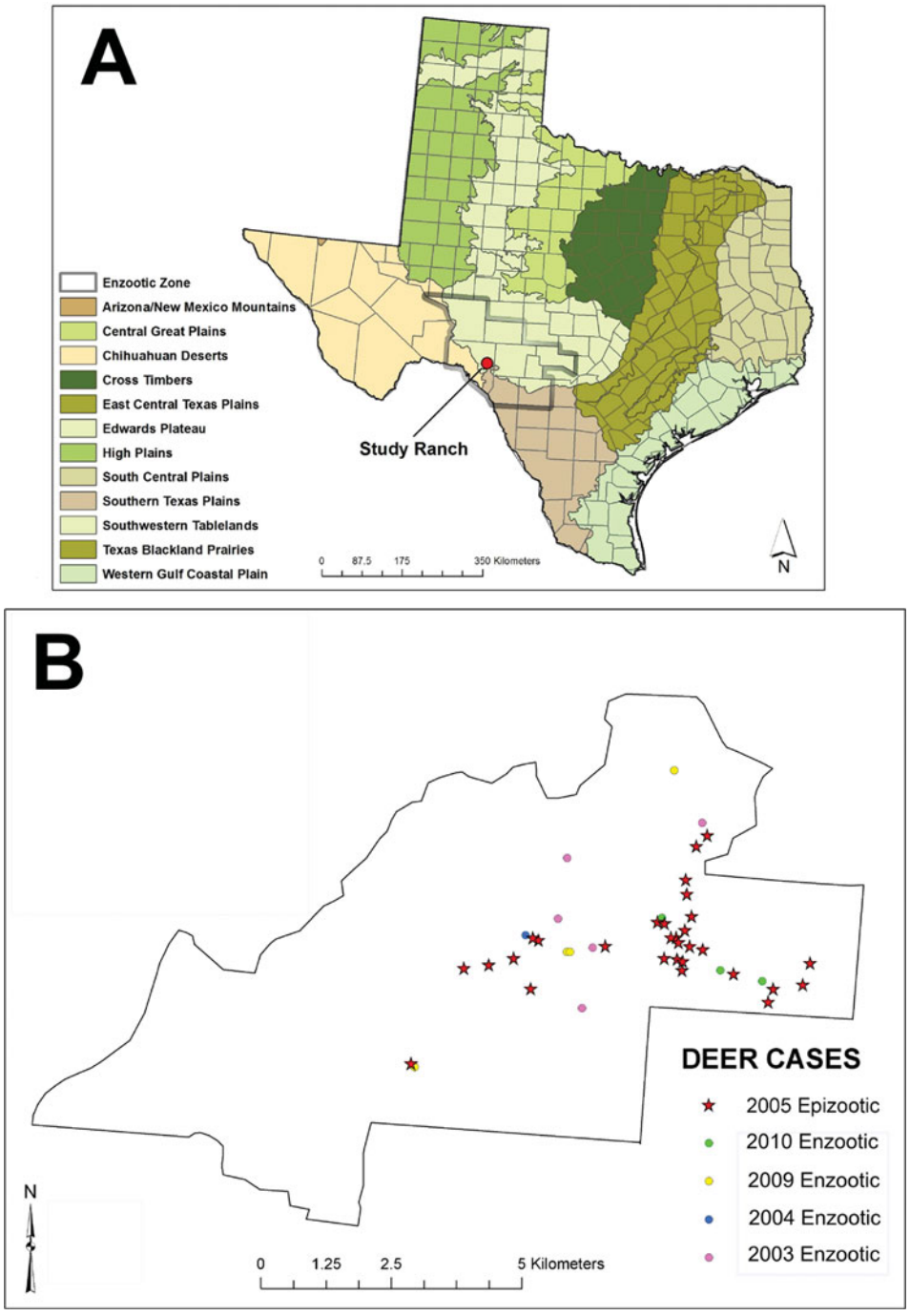

Figure 1. (A) Location of the study ranch in west Texas where fly trapping was conducted during the 2005 summertime anthrax season. Colors represent the twelve Level III ecoregions in Texas following Omernik (1995). The ranch is situated on the western edge of the Edwards Plateau. The fuzzy boundary (dark gray) represents the current estimated extent of the west Texas enzootic zone to the nearest county based on outbreak reports from 2000–2012. (B) The spatial pattern of dead deer associated with anthrax from 2003 through 2010 based on field observation and laboratory testing. (Color figure available online.)

vultures, *Cathartes aura*, circling overhead to find carcasses. This proved to be a most effective method for searching out carcasses as reported elsewhere in the literature (Turnbull et al. 2010).

Anthrax Diagnostics for Deer Cases

Biological samples have been collected from deer carcasses since 2003. During the 2005 epizootic, and in the weeks following, tissue samples and small bone fragments were collected from several animal carcasses and shipped to MRIGlobal in Palm Bay, Florida, and the U.S. Naval Medical Research Center in Silver Spring, Maryland. Several carcasses were burnt immediately on finding them as part of ranch-wide control efforts and samples were not collected. Classical microbiology and polymerase chain reaction (PCR) were used to diagnose anthrax from those carcasses sampled (Blackburn

Table 1. Population estimates, anthrax case numbers (culture positive and untested suspect cases), and herd mortality rates with 95 percent binomial exact confidence intervals for a white-tailed deer, *Odocoileus virginianus*, herd in west Texas

Year	Anthrax cases	Deer population	Prevalence	Lower 95% CI	Upper 95% CI
2001	100	1,358	7.3638	0.0603	8.8841
2002	0	888	0.0000	0.0000	0.4146
2003	7	1,059	0.6610	0.0027	1.3571
2004	1	1,338	0.0747	0.0000	0.4157
2005	42	1,332	3.1532	0.0228	4.2384
2006	0	1,300	0.0000	0.0000	0.2834
2007	0	1,584	0.0000	0.0000	0.2326
2008	0	1,666	0.0000	0.0000	0.2212
2009	4	1,672	0.2392	0.0007	0.6114
2010	2	1,641	0.1219	0.0001	0.4396

Note: CI = confidence interval.

et al. 2010). Briefly, materials were either heat shocked (thirty minutes at 700°C) or suspended in 100 percent ethanol for one hour prior to plating. Samples were concentrated by centrifugation and the alcohol was replaced with molecular grade water. The pellet was suspended in the water then streaked on sheep blood agar and ACTSBA media for primary isolation of *B. anthracis*. Gram stains of nonhemolytic colonies showing long chains of large gram-positive rods were presumptively identified as *B. anthracis*. Presumptive colonies of *B. anthracis* were subcultured for susceptibility to gamma phage lysis and for DNA extraction for PCR. Laboratory results were linked to the spatial data in ArcGIS v. 10 (ESRI 2010). In this report, we mapped laboratory-confirmed positives and presumptive suspect cases. Laboratory negatives were removed from the spatial analyses. For spatial analyses, all suspect and culture-positive cases were assumed to be anthrax cases and treated as a single sample size.

Deer Population Estimates and Anthrax Mortality Calculations

Ranch management staff provided deer population estimates derived from state regulated spotlight counts (Jester and Dillard 2010) and helicopter surveys. Total population estimates were provided from 1995 to 2010. Additionally, spatially explicit surveys for anthrax cases were available from 2003 to 2010. Ranch-wide mortality estimates were calculated for 2001 through 2010 using the number of carcasses identified and total deer population for that year. The 95 percent exact binomial confidence intervals were calculated for mortality estimates using the epitools package in R (Aragon, Fay, and Wollschlaeger 2012).

Data on the spatial distribution of the deer herd across the ranch were available for the 2003–2004 population estimates. Ranch staff used expert opinion and data from game cameras used throughout the 2003 summer period to estimate the number of bucks and does that use each protein feeder setup on the ranch during that period. These data were used in a kernel density estimation (KDE) to illustrate the distribution of the deer herd across the ranch.

Average Nearest Neighbor Analysis of Deer Carcass Locations

To determine whether anthrax cases clustered on the ranch, we used the average nearest neighbor index (ANNI). The ANNI is a ratio of the average distance between each anthrax case and its nearest neighbors compared to a random distribution of neighbors. If the ratio is less than 1, the pattern is clustered; if the ratio is greater than 1, the pattern is dispersed (Clark and Evans 1954). We used the ANNI tool in ArcGIS 10 with Euclidian distances and area of the ranch in square meters.

Kernel Density Analysis of Deer Carcass Locations and the Deer Population

Kernel density analysis was used to identify hotspots of deer carcass locations. KDE is a technique for calculating weighted densities of events over a gridded surface within a kernel, or spatial filter. KDE was performed with the Spatial Analyst Extension for ArcGIS 10. ArcGIS employs the quadratic kernel function described in Silverman (1986, 76, Equation 4.5). This function was used to estimate carcass densities. Outputs are raster surfaces of smoothed density values of carcasses across the study area. We use the maximum step length (1,259 m) or the maximum distance that a radio-collared deer traveled in any two consecutive relocations (Table 2) as the bandwidth and 100-m gridded outputs. Radio telemetry data were available from seven deer radio tracked from July through October 2005 during the fly trapping study (Blackburn 2006). Step lengths were calculated using Geospatial Modeling Environment v. 0.7.2.0 (Beyer 2010). Hotspots for carcasses were defined as the upper 25 percent, 10 percent, and 5 percent of estimated density values following Nelson and Boots (2008).

Table 2. Shortest, average, and maximum travel distance (step length) between consecutive relocations of VHF radio-collared white-tailed deer during the summer anthrax risk period, 2005

Deer	Shortest step length (m)	Average step length (m)	Maximum step length (m)	*n*
1	0	394.1	1,189.1	39
2	113.4	473.3	1,221.5	26
3	3.9	482.1	1,299.2	25
5	46.3	198.5	441.7	39
6	121.6	650.6	2,209.8	25
8	0	294.1	990.1	28
9	0	272.1	1,461.5	27
Herd average	40.7	395.0	1,259.0	

Note: VHF = Very high frequency. Original telemetry data from Blackburn (2006).

We used the same KDE parameters to estimate the spatial distribution of the ranch deer herd using the 2003–2004 feeder population estimates. The total population estimate from the feeder counts was similar to the 2005 population estimate and used in the KDE analysis, as it was spatially explicit (deer counts were assigned to specific coordinates).

Spatial Mortality Estimates: Dual Kernel Density Analysis of Anthrax Cases Relative to Deer Population

We used a dual kernel density approach to evaluate the spatial patterns of anthrax mortality. Following Kelsall and Diggle (1995), we used the natural log of the ratio of the deer carcass kernel surface and the 2003–2004 population kernel surface to estimate areas of high anthrax mortality on the ranch.

Hematophagous Fly Collecting

We implemented a spatially explicit sampling design to evaluate the spatial and temporal patterns of biting flies relative to known areas of deer anthrax on the ranch. Biting fly sampling was conducted over three periods between 5 June and 21 August 2005 using seven unbaited Nzi fly traps (www.nzitrap.com; Figure 2). The Nzi is a nylon and polyester net trap with countershaded blue and black panels designed to attract flies through black body attraction (Mihok 2002). The top of the trap is translucent net mesh that allows natural light in. Once a fly enters the trap, it flies upward to a small opening in the net and into a series of two-liter trap bottles, eventually ending up in a plastic catch bag (Figure 2 inset). Traps were set up for approximately twenty-four hours at each sampling site. Forty-two sampling sites were selected prior to the collection period to represent the geographic area and diversity of habitats across the ranch. Sites were placed in proximity to unpaved ranch roads to facilitate rotation from day to day by an all-terrain vehicle (ATV) across the relatively large ranch. The order of sites was randomly selected for the first setup period and then repeated throughout the sampling season to ensure the time periods and spatial

Figure 2. Unbaited Nzi fly trap setup. Flies enter through the opening between the black patches, fly upward toward the light, and get trapped in the bottles. The inset shows flies trapped in the bottles and in the catch bag. (Color figure available online.)

locations were represented near equally. At the end of each trap event, individual sample bags of flies were frozen at –20°C until they could be sorted and counted. Temperature, humidity, and wind speed were collected at the start and end of each sampling event using a handheld digital meter (Kestrel Instruments Model 4100, Neilsen-Kellerman, Boothwyn, PA). Wind speed readings were averaged for one minute and recorded as meters per second. Wind direction was measured with a handheld wind vein and recorded in degrees between 0 and 360 using a handheld compass. In addition to environmental conditions measured in situ, elevation for each site was derived from a U.S. Geological Survey 30-m digital elevation model (DEM). Normalized difference vegetation index (NDVI) values were extracted for each trap site from 250-m resolution MODIS data downloaded from the Global Land Cover Facility (www.landcover.org).

Given the observed diversity of fly species capable of transmitting *B. anthracis*, total fly counts were pooled for this study. Flies were thawed; sorted into hematophagous flies, necrophagous flies, and other insects based on head and body morphology (e.g., eye shape, mouth part shape) following methods described by Goodwin and Drees (1996); and counted as total numbers. To account for variation in fly numbers associated with differences in sampling event lengths, each fly count was divided by the total time the trap was set out uninterrupted. Therefore, fly catch rates were defined as:

$$\text{Catch rate} = \frac{\text{Total biting flies captured}}{\text{decimal hours of the trapping event}}$$

Descriptive statistics were calculated for each of the continuous environmental variables for the forty-two sampling sites for each setup period. A total of four setup periods were completed during the sampling season; however, Setup 4 was an additional set of traps within the time period of Setup 3 and therefore both represent a single time period.

Statistical Analyses

Correlation. Shapiro-Wilk tests of normality were run on all catch rates from all trap sites. Spearman's rho correlations were calculated for catch rates and the environmental variables collected in situ and the DEM and NDVI. Statistical analyses were performed in SPSS v. 20 (IBM, Inc. 2012).

Soil Analysis. Although *B. anthracis* is a soil-borne pathogen, it is not found evenly across all soil conditions (Dragon and Rennie 1995; Smith et al. 2000; Dragon et al. 2005; Hugh-Jones and Blackburn 2009). Historically, Van Ness and Stein (1956) defined the geographic range of anthrax in the United States by soil type, arguing that high soil pH (alkaline soils with high pH values) and high calcium, nitrogen, and organic content were the most important factors for spore survival. This study followed the ranges of pH and soil conditions summarized by Hugh-Jones and Blackburn (2009). Soil samples were collected at each fly trap location. Soil samples were shipped to the Soils Lab at the Louisiana State University Agricultural Center. Samples were processed for soil pH and eight additional minerals (calcium, copper, magnesium, phosphorus, potassium, sodium, sulfur, zinc). Soils analysis followed published protocols available online at http://www.lsuagcenter.com/en/our_offices/departments/SPESS/ServiceLabs/soil_testing_lab/procedures/Procedures+Used+at+the+Laboratory.htm). To map soil conditions for this study, we interpolated soil values using inverse distance weighting in the spatial analysis routine using square distance and twelve neighbors. In this study, soil pH was mapped, as it is arguably a limiting factor broadly controlling the range of viable *B. anthracis* spores (Smith et al. 2000; Hugh-Jones and Blackburn 2009).

Centrographic Statistics of Fly Catch Data. The spatial mean, standard deviation ellipse, and standard distance were calculated for catch rates at each trap site and each trap setup period using the spatial statistics toolbox in ArcGIS. Each test was run for trap locations and again weighted by fly catch rate. The unweighted spatial mean and standard deviation ellipse was used to determine whether the trap sites reflected the larger geography of the ranch or were biased toward one region of the ranch or another. It was important to test the hypothesis of fly densities varying across the ranch from west to east similar to anthrax case data. The weighted standard distance values were used in the bandwidth calculation for KDE (see Hotspot Analysis of Fly Data Using Kernel Density Estimation).

Hotspot Analysis of Fly Data Using Kernel Density Estimation. KDE was also used to plot fly densities (standardized catch rate values) for each of the setup periods. This function was used to estimate fly densities using fly catch rates as the weight. Outputs are raster

surfaces of smoothed density values of biting fly catch rates across the study area. For biting fly data we used the optimal bandwidth function provided in Fotheringham, Brunsdon, and Charlton (2000):

$$h_{opt} = \left[\frac{2}{3n}\right]^{(\frac{1}{4})} \sigma,$$

where n is the sample size (here fly trapping locations) and σ is the standard distance of the trap locations. Standard distance was calculated with the spatial statistics toolbox in ArcGIS 10. To compare fly density surfaces, we calculated h_{opt} for each of the four time periods and averaged the values (Nelson and Boots 2008). The output grid cell size was set to 100 m for all analyses, matching the output of deer carcass density surfaces.

As with the KDE analyses for deer, hotspots for hematophagous flies were defined as the upper 25 percent, 10 percent, and 5 percent of estimated density values of catch rates following Nelson and Boots (2008). Hotspots were defined separately for each given setup period expressed in a distribution for all sampling sites for the time period. Therefore, a hotspot in any one setup period is only reflective of that single period and not reflective of fly occurrence in pre- or postsetup periods.

Getis–Ord Spatial Clustering of Biting Flies. The Getis–Ord statistic $G_i^*(d)$ (Getis and Ord 1992; Ord and Getis 1995) was used to determine whether significant local spatial clustering occurred during the study period. The $G_i^*(d)$ statistic tests for local spatial clusters in group-level data and assesses the association of the variable of interest within a set distance of each observation in the data set tested (Jacquez et al. 2002). The $G_i^*(d)$ statistic is written as:

$$G_i^*(d) = \frac{\sum_j w_{ij}(d)\, x_j}{\sum_j x_j},$$

where x_j are the catch rates of flies, and w_{ij} is a binary (0, 1), symmetric weights matrix with 1 for all j within distance d of point i with all other values being 0.

The $G_i^*(d)$ was calculated for the hematophagous fly catch data set using the Spatial Statistics Toolbox in ArcGIS v10. Three distances (d) were selected for this study. The smallest distance, 500-m, was selected to capture localized catch rates, representative of individual trap sites and nearest neighbors. Distances were then set in 500 m increments to 1,500 m. The largest distance (1,500 m) was selected to capture a greater percentage of the study area in each calculation. As the G_i^* values are normal variants of the z distribution, only those G_i^* values greater than 1.96 were considered significant. Following Getis et al. (2003), cluster membership was defined by significant G_i^* values at the distance with the maximum G_i^* score. For a trap site to remain a member of a statistically significant cluster from one distance to another, the G_i^* value must increase from test distance size to test distance size. If the G_i^* value did not increase with distances, although G_i^* values might be statistically significant, they were not considered members of clusters. Getis and Aldstadt (2004) defined this as the critical distance, d_c. All clusters presented in this study were defined at d_c. The G_i^* can be evaluated as a two-tailed test with negative values representing clusters of low values (Getis and Ord 1992). Maps were produced to illustrate the spatial distribution of biting fly clusters for each setup period (1–3). The sample size of Setup 4 was too small for this analysis and was excluded. Anthrax positive and suspect carcass locations were overlain to show the relative distribution of disease cases relative to fly clusters.

Results

Deer Population Data and Anthrax Mortality Rates

A large anthrax outbreak occurred in 2001 and ranch management estimated more than 100 dead animals (Table 1). Although the carcasses were not mapped individually with GPS, cases were concentrated in the central and eastern areas of the ranch with no carcasses identified in the west. The 2005 acute outbreak investigated in detail in this study occurred late in the summer, with cases first being found in late August early September (first confirmed case found 6 September 2005) and the last case being found in mid-October. There was, however, at least one early deer case in June 2005 in the central area of the ranch, in which necrophagous flies were confirmed to be positive for *B. anthracis* (Blackburn et al. 2010). The acute outbreak coincided with laboratory-confirmed cases throughout Val Verde County. No large outbreaks occurred between 2001 and 2005 on the ranch; however, ranch managers and researchers confirmed between one and seven cases per year in the interim periods (Table 1). The distribution of carcasses found between 2003 and 2005 was concentrated in the central and eastern portions of the ranch (Figure 1B), with cases in 2009 and 2010 also occurring in the areas impacted in 2005. Although the 2001 outbreak was not GPS mapped, the

2003 to 2005 distribution coincides with the distribution of those cases according to the ranch staff that disposed of carcasses. Anthrax mortality rates and corresponding 95 percent exact binomial confidence intervals for 2001 through 2010 are summarized in Table 1.

ANNI and Hotspot Analysis of Deer Carcasses and Deer Population

The ANNI confirmed that the thirty-one anthrax carcasses with GPS coordinates were highly spatially clustered across the study area (observed mean distance = 336.9 m, expected mean distance = 767.7 m, nearest neighbor ratio = 0.439, z score = –5.97, $p < 0.0001$).

Kernel density estimates of anthrax cases identified a hotspot in the east central portion of the ranch along the dry river bed in proximity to the hotspots identified for biting flies (Figure 3A). In contrast, the KDE for the deer population was widely distributed across the ranch, with concentrations in eastern and western portions of the ranch (Figure 3B).

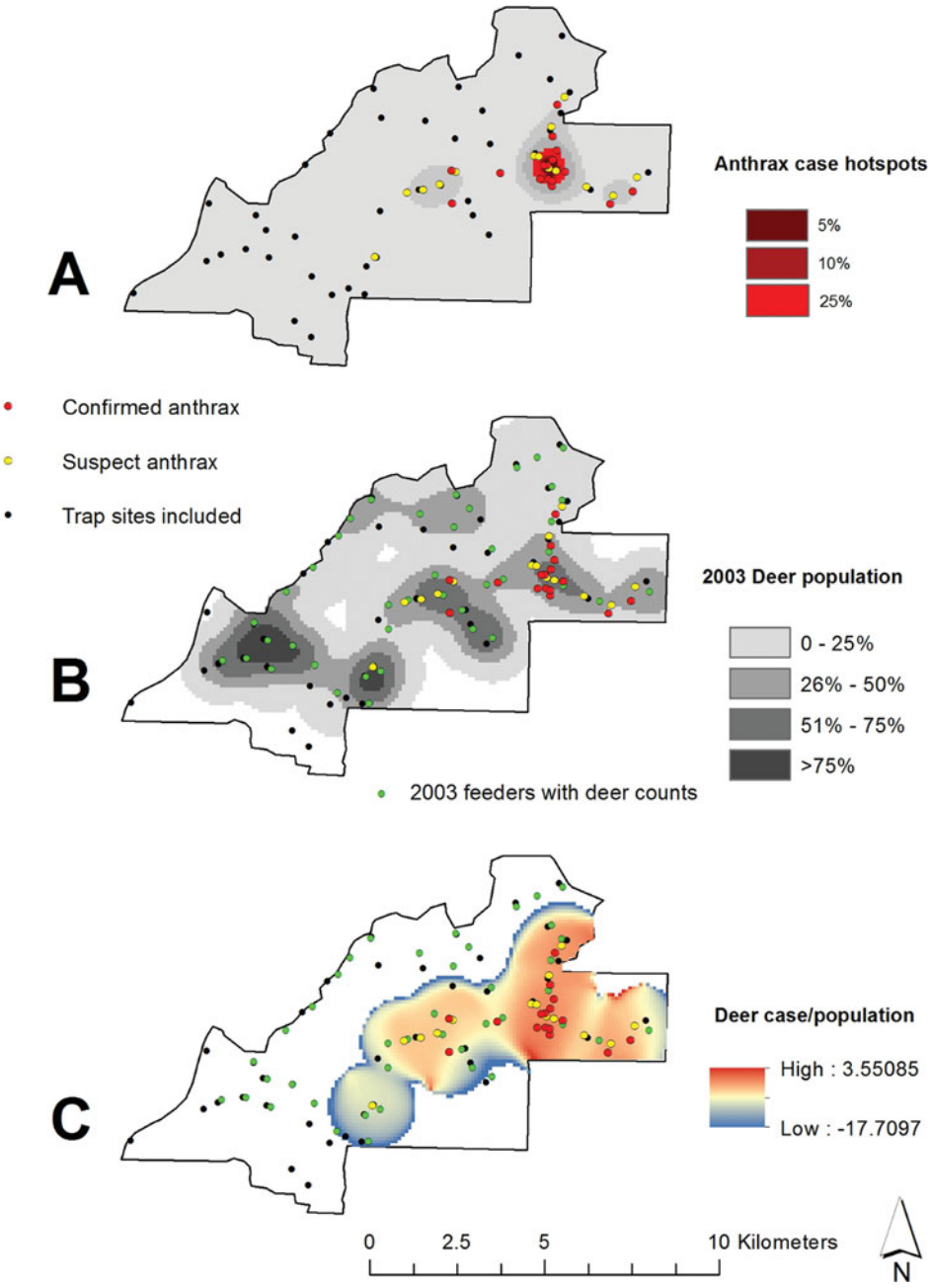

Figure 3. (A) Kernel density estimation of deer anthrax during the 2005 epizootic. Red color ramp represents the 5 percent, 10 percent, and 25 percent hotspot cutoffs. Yellow dots represent suspect anthrax cases, red dots represent confirmed anthrax cases, and black dots represent fly trap locations for the 2005 trapping season. (B) Kernel density estimation of the deer population across the ranch based on population estimates during the 2003–2004 deer assessment surveys. Deer counts were assigned to protein feeding stations (green dots). (C) Dual kernel density estimation of deer anthrax mortality using estimates from A and B. Yellow to red on color ramp indicates areas of higher anthrax density. (Color figure available online.)

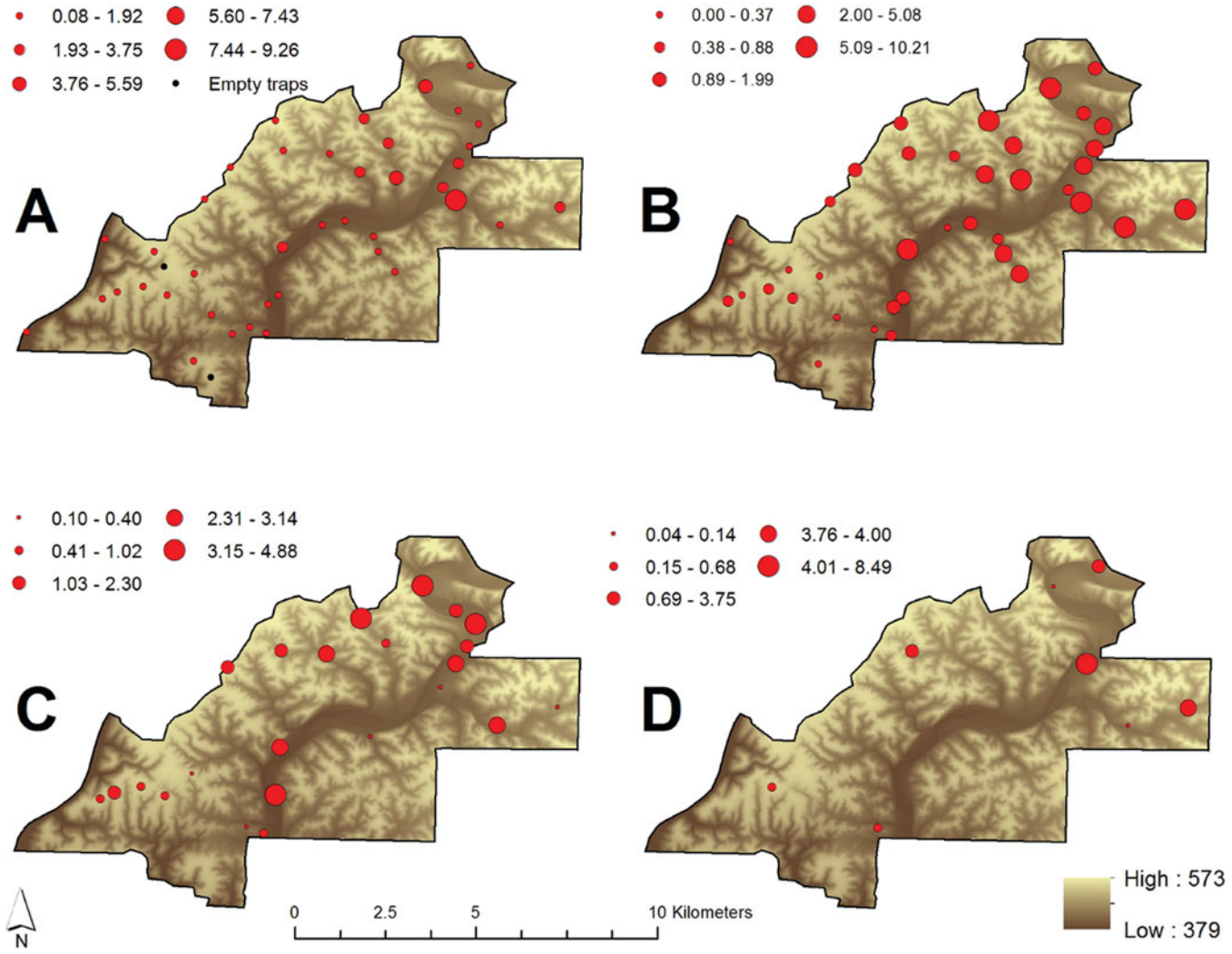

Figure 4. Graduated symbols representing the fly catch rates at each trap site where traps were run during (A) Setup 1, (B) Setup 2, (C) Setup 3, and (D) Setup 4. Base map is elevation in meters with darker areas being lower in elevation. (Color figure available online.)

Dual Kernel Density Estimates

Dual KDE for anthrax risk using the natural log ratio of deer carcasses to the deer population identified the eastern portion of the dry river bed as the region of highest case rates or greatest risk for anthrax (Figure 3C).

Fly Trap Data and Centrographic Statistics

A total of 5,114 biting flies were collected during 113 uninterrupted trapping events. Setup 1 spanned the period from 5 to 11 June 2005 and included forty sampling sites. Setup 2 spanned 10 to 16 June 2005 and included forty sampling sites. Setup 3 spanned 12 to 21 August 2005 and included twenty-three sampling sites. Setup 4 represents a subsample of sites sampled again on 15 August 2005 (a time period when multiple deer carcasses were being found, potentially reflecting the start of the epizootic). Fly catch rates per trap site are illustrated with graduated symbols in Figure 4. The spatial mean and standard deviation ellipse of unweighted trap locations indicates that fly trap sites were evenly distributed across the ranch and not biased toward any one side of the ranch (Figure 5A). Centrographic statistics for fly trap locations and weighted by fly catch rates for each trap period are presented in Figure 5B. Weighted centrographic statistics suggest that biting flies were more heavily concentrated in the eastern trap locations throughout the sampling period (Figures 5B, 5C).

Statistical Analyses

Catch rate data were not normally distributed based on the Shapiro-Wilk test (test statistic 0.695, $p =$

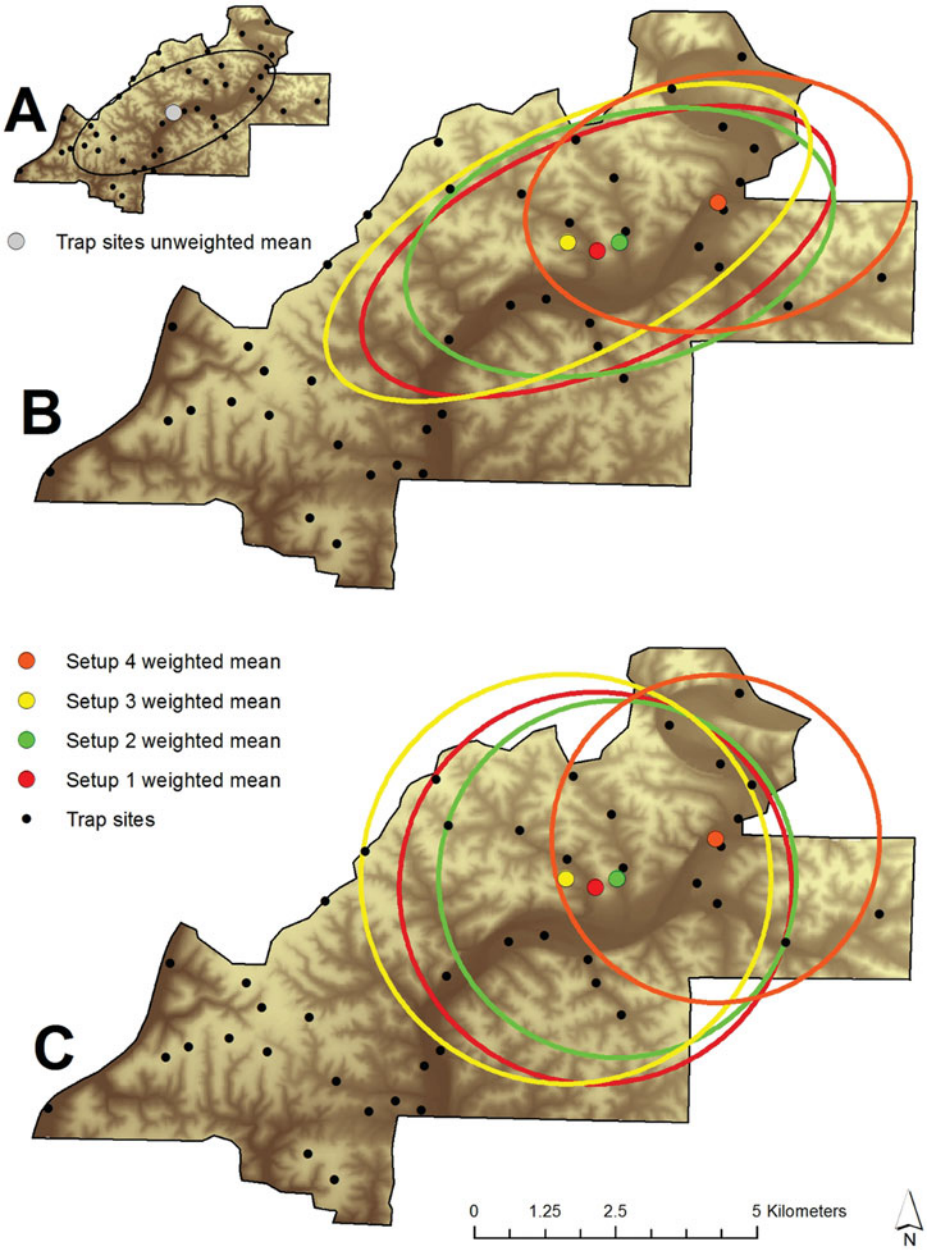

Figure 5. (A) All forty-two Nzi trap locations across the study ranch (black dots); the unweighted spatial mean of all trap sites with one standard deviation ellipse based on unweighted trap locations suggesting that the traps were relatively evenly spatially distributed. (B) Catch rate weighted spatial means and single standard deviation ellipses for each of the four trapping periods on the study ranch. (C) Weighted spatial means and standard distance circles for each setup period. Weighted means reflect an eastern bias in fly catch rates on the ranch. Base map is a 30-m resolution raster of altitude in meters. (Color figure available online.)

0.000). Spearman's rho identified a weak but significant positive correlation between fly catch rates and NDVI (0.216, $p = 0.022$). Weak but significant correlations were also identified between catch rate and altitude (–0.215, $p = 0.022$) and average wind speed (averaged wind speed from the setup and pickup measurements; –0.261, $p = 0.005$).

Hotspot Analysis: Biting Flies. Kernel density estimates identified a pattern of biting fly hotspots across the fly trapping periods in the northeast and east central portion of the ranch along the Dry Devil's River bed and valleys that lead to it (Figure 6). A hotspot along the eastern central property boundary of the ranch was present across the trap setup periods. The areas of highest density were in proximity to or had apparent overlap with the distribution of both positive and suspect cases, with both hotspots and carcass locations dominant on the eastern areas of the ranch. No hotspots of high fly catch rates occurred on the western side of the ranch during the 2005 sampling year.

Figure 6A illustrates biting fly hotspots plotted over an interpolated surface average wind speed. There is clear delineation between high wind speeds on the

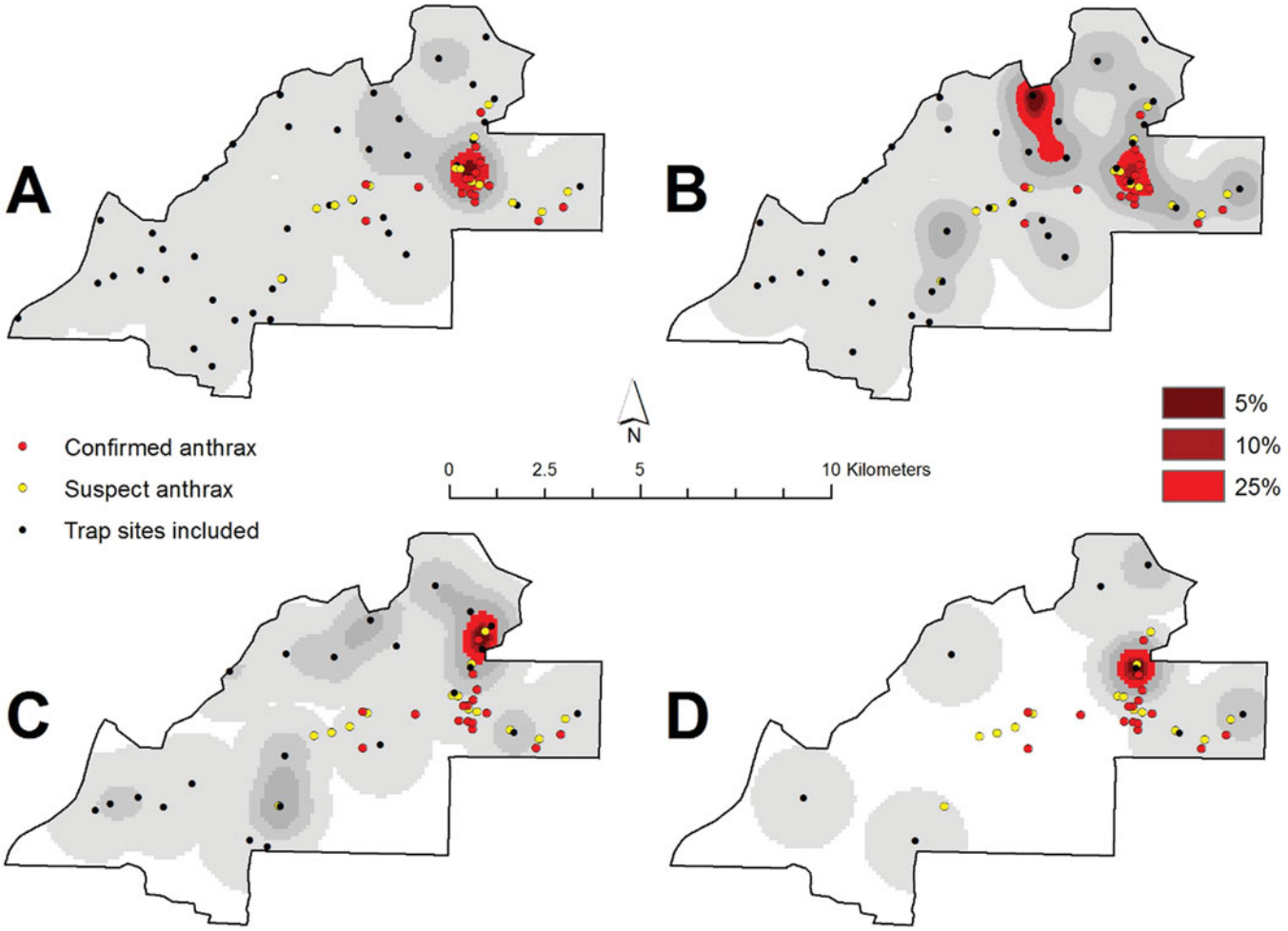

Figure 6. Kernel density estimates of tabanid fly catch rates for (A) Setup 1, (B) Setup 2, (C) Setup 3, and (D) Setup 4. Red color ramp indicates the 25 percent, 10 percent, and 5 percent of the highest values in each setup period. Gray color ramp reflects density estimates less than the 25 percent cutoff. Red dots indicate spatial locations of laboratory-confirmed anthrax mortality in deer, and yellow dots reflect deer carcasses suspected of anthrax but not tested, as carcasses were destroyed without sample collection during outbreak response. (Color figure available online.)

ridgeline along the northwest border of the ranch and the eastern river valley where wind speeds were lower. Kernel density estimates were overlain to demonstrate the relationship between wind speeds and fly catches. Although the correlation value was weak, low wind speeds were significantly correlated with high catch rates, indicating that wind speeds might deter flies in the western regions of the ranch. Figure 6B displays the kernel density hotspots overlain on the vegetation classification derived from the LandSat data. In general, hotspots occurred in low-lying areas with dense vegetation centered on a large river bed, which was corroborated by the positive correlation with catch rate and NDVI. Although nearly all trap locations had flies during at least one single sampling event in this study, the highland areas of the west typically had fewer than ten totals flies per trapping event. A weak negative correlation was found between catch rates and altitude. In contrast, total catches on the eastern side of the ranch were often greater than 100 flies. In addition to having less vegetation coverage in the upland areas, wind speeds were generally higher in these areas. Lowland canyons did provide shelter during periods of high winds, often with wind speeds nearly an order of magnitude higher upslope.

Figure 6C illustrates soil pH across the trap sites. This is interesting from a microbial-centric point of view, as soil characteristics will dictate the long-term survival of *B. anthracis* spores. In general, soil pH was relatively high at all sampling sites with no clear delineation of regions with soil pH unfavorable for *B. anthracis*.

Getis Analysis of Fly Trap Data. The G_i^* statistic identified local clusters of high catch rates in each of the

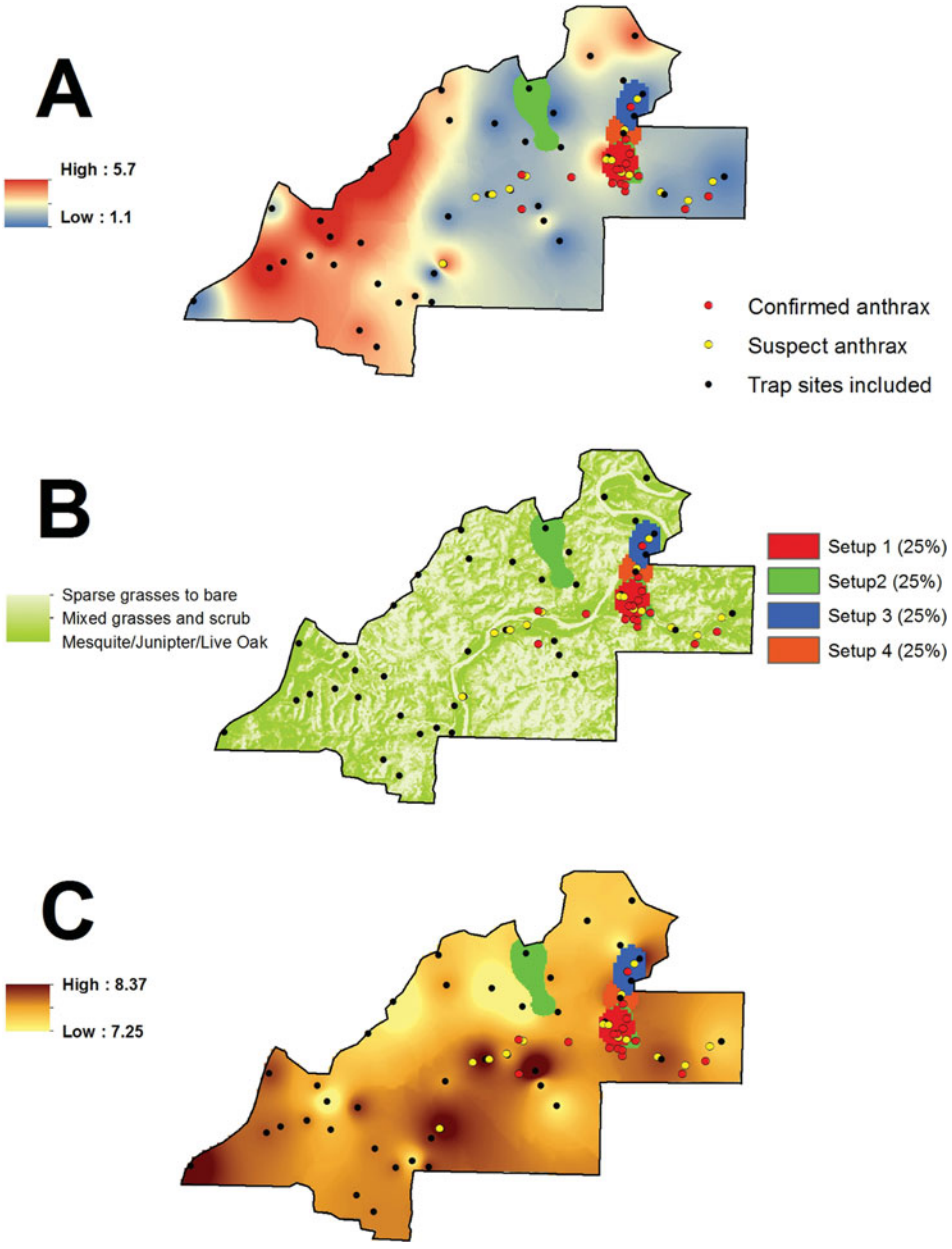

Figure 7. Highest 25 percent cutoff values of biting fly catch rate kernel density estimates for each setup period plotted over average wind speed from (A) in situ measurement, (B) a six-class habitat map derived from Landsat 7 TM+ imagery, and (C) soil pH from samples collected at each trap site. Carcass sites are indicated by red dots (confirmed cases) and yellow dots (suspect cases). Black dots are fly trap sites. (Color figure available online.)

three setup periods examined. The highest G_i^* values for high catch rates at each of the periods was at $d_c = 500$ m, with Setup 1 also having two clusters at 1,000 m. Clusters of low values were detected at 500 m for Setup 1 but not for the other two periods. Figure 7 illustrates the distribution of clusters for both low and high values at defined critical distance thresholds. The G_i^* clusters generally agree with the kernel density results for Setup periods 1 and 2, and both indicate that the northeast central region (low-lying areas) promotes high numbers of flies, whereas the northwestern (upland) areas did not. In fact, the highland areas were identified as clusters of significantly low catch rate values in Setup period 1. Additionally, G_i^* and critical distance indicated localized clusters of high numbers of flies around trap sites.

Discussion

In this study, we studied spatial patterns of deer anthrax on the Edwards Plateau in west Texas. We related these spatial patterns to biting fly densities, evaluating the potential for biting flies to serve as mechanical vectors during anthrax outbreaks. Toward these questions we applied several spatial analytical approaches and spatial statistics. These are summarized in Table 3.

Table 3. Summary of spatial analytical and statistical analyses employed to relate deer anthrax mortality and biting fly densities to a white-tailed deer herd of the Edwards Plateau in west Texas

Hypothesis	Spatial analytical approach	General findings
Anthrax mortality events are spatially concentrated	Kernel density analysis	Deer cases were highly concentrated in areas of dense vegetation in the eastern half of the study area
Anthrax mortalities are spatially clustered	Average nearest neighbor index	Deer carcasses were significantly clustered on the landscape
Deer anthrax mortality was limited to a portion of the total herd	Dual kernel density analysis	Deer cases were highly concentrated in the east despite a broad distribution of deer across the study area
Biting flies were spatially concentrated across the risk period	Time-specific kernel density analysis	Biting flies were concentrated in proximity to anthrax locations across all time periods
Biting fly density was associated with ecological conditions	Nonparametric correlation	Biting fly density was correlated with high NDVI, low elevation, and low wind speeds
Biting flies were spatially clustered	Getis and Ord statistic	Biting flies were spatially clustered at local distances on the eastern half of the ranch

Note: NDVI = normalized difference vegetation index.

Few anthrax studies have provided in-depth outbreak records for a single, well-studied animal herd over multiple years or at high spatial resolution within an outbreak. In this study, we provided a ten-year history of anthrax in a white-tailed deer herd from a large study ranch positioned along the western edge of the Edwards Plateau within the west Texas anthrax enzootic zone. We broadly define the geographic boundaries of that zone at the county level using available observations and historical records reviewed by Blackburn (2006). Although anthrax is classically thought of as an acute disease with high mortality rates, the annual mortality rates presented here identify a more dynamic disease cycle with severe acute epizootics with high mortality in some years (2001, 2005) and enzootic cases in other years (2003, 2004, 2009, 2010). Confidence intervals suggest that enzootic cases are likely in all years. The limited number of carcasses found in any year surely underestimates the disease, as carcasses are difficult to find and spotters rely heavily on vultures as proxies for cases. In a recent study of zebra anthrax in Etosha National Park, Namibia, Bellan et al. (2013) estimated that 3.8 times more zebra died from anthrax than were found in a given epizootic year. Across years, epizootic or enzootic, anthrax cases have only been documented on the central and eastern areas of the ranch and not in the west. We used a dual kernel density analysis to map anthrax mortality rates of the 2005 epizootic based on deer carcass data and illustrated these regions, in particular the confluence of a large seasonally dry river bed and several smaller canyons that feed into it in the eastern central region of the ranch. A highly significant ANNI value confirmed that deer carcasses were closely clustered on the landscape. Broadly, these results suggest that the pathogen is active in the herd annually, with strong evidence for localized transmission.

The second objective was to determine whether areas of high anthrax mortality also supported high biting fly populations during the anthrax risk period. Biting flies have been implicated in anthrax outbreaks nearly worldwide. Hotspot analysis using KDE clearly identified areas in the east central and northeastern portions of the ranch with high fly catch rates across each of four sampling periods during the 2005 summer leading up to the acute anthrax outbreak. The Getis G_i^* analysis also illustrated significant clusters of high fly catches on the eastern half of the ranch at 500 m and 1,000 m in the first sampling period and 500 m in the subsequent periods. As the test is two tailed, it also identified meaningful cold spots or an absence of biting flies on the western side of the ranch during the first trapping period. Although there were no other significant low value clusters identified in later sampling periods, graduated symbols of fly catch rates (Figure 4) confirm that catch rates were very low, sometimes even a single biting fly in twenty-four hours, across the western sampling sites. This was likely due in large part to the higher elevation, shorter sparse vegetation, and overall higher wind speeds on the western side (Figure 7). In contrast, wind speeds were relatively low, water more abundant, and vegetation more dense along the river bed and associated habitat of the eastern half of the ranch. Although correlations were weak, the Spearman's rho values confirmed significant negative relationships between fly catch rates,

altitude, and wind speed and a positive relationship between catch rates and NDVI, supporting these hypotheses. In an earlier study of *T. nigrovittatus*, biting fly activity was correlated with warm temperatures (around 25°C) and windless conditions (Dale and Axtell 1975).

These spatial techniques have been used for other disease systems to associate areas of high vector density with case locations. Jeffery et al. (2002) used a combination of kriging and the G_i^* statistic to identify areas of abundant mosquito vectors for Ross River and Barmah Forest viruses in Australia. That same study found agreement between both techniques for identifying spatiotemporal hotspots of insect populations and used the G_i^* statistic to identify clusters of both high and low catch values across weekly sampling intervals. Getis et al. (2003) also found spatiotemporal clustering at local scales for mosquitoes associated with Dengue fever in Peru. Vazquez-Prokopec et al. (2005) and Cecere et al. (2004) identified significant clusters of high Triamtomine insect populations in rural Argentina using the G_i^*. Similar to this study, maximum G_i^* values were at local distances, suggestive of limited individual insect dispersal despite potential for long-distance movements (over several kilometers). In this study, clustering of high fly catches was greatest (highest G_i^* values) at a critical distance of 500 m, suggesting that concentrations of flies were highly localized. This could be an artifact of the sampling scheme, with some trap locations spaced farther apart than others. This also makes biological sense, however; Foil (1989) provided strong evidence that despite the potential for long-distance movements in biting flies, most Tabanids in mark–recapture studies traveled less than 50 m and often returned to the same host for multiple feedings. Although these results do not preclude flies from traveling several kilometers, it is probable that the flies will remain within a short distance of the deer. Deer counts by ranch staff indicate a high number of deer on the eastern end of the ranch, suggesting shorter travel distances between meals for individual flies. This is not to suggest that deer are absent or not using the western side of the ranch. As was illustrated by the KDE of deer population, the herd takes advantage of much of the ranch (Figure 3). Although there was some shifting of local fly clusters around individual trap sites over the season, a core area was identified along the large dry river bed running through the north central portion of the ranch. This area was also identified by the kernel density analyses (Figure 6). This area had the densest vegetation and was at low altitude, providing hosts to feed on and refuge from high winds found in the northern upland areas (Figure 7).

The clustering patterns of this study indicate higher fly numbers at short distances. No data are available, however, on the flight distances of flies on this ranch. Although Foil (1989) indicated that flies return to individual host animals for repeat blood meals, the study also indicated that flies can travel upwards of 200 m in search of a meal. Likewise, wind speeds did show variability and high valley winds could potentially force flies to travel great distances. Sheppard and Wilson (1976) used mark recapture methods to study the flight ranges of female tabanids in a Louisiana hardwood forest and reported that biting flies were captured from 0.8 to 6.8 km away from the release site, with a fairly uniform distribution of recaptures across distances, although the greatest numbers were captured within 0.8 km, the closest distance to the release site. Trap locations in open areas at least 90 m from the wooded sites had higher recaptures. In this study, the eastern area of the ranch had relatively taller vegetation, but all trap sites were open and all traps were > 2 m from tall vegetation, although ecologically this landscape is very different from a hardwood forest.

A parallel telemetry study of white-tailed deer movements on the ranch confirmed that individual deer have limited seasonal home ranges during the anthrax risk period whether tracked on the western or eastern half of the ranch (Blackburn 2006). Likewise, the step length analysis of deer radio telemetry data performed for this study indicated that deer might travel as little as 40 m in a single movement event (or less in one case; Table 2) during the summer period. This suggests that deer do not use long-distance movement to avoid flies. No data are available on potential avoidance behaviors in this deer population, though. A combination of environmental conditions, deer avoidance behaviors, and 50-m or greater search patterns by flies could quickly expand the spatial footprint of an outbreak if flies were exposed to anthrax bacilli during blood meals. This can be defined as the *space-multiplier hypothesis* (Hugh-Jones and De Vos 2002).

Foil (1989) defined a single fly's tenacity to return the same host when interrupted as *feeding persistence*. High feeding persistence would decrease the likelihood of transmission between hosts but, conversely, host jumps might increase with host density (Foil 1989). The spatial analysis from this study identified clusters of biting flies proximal to or overlapping areas of relatively high deer density. In this study, we limited our analysis to

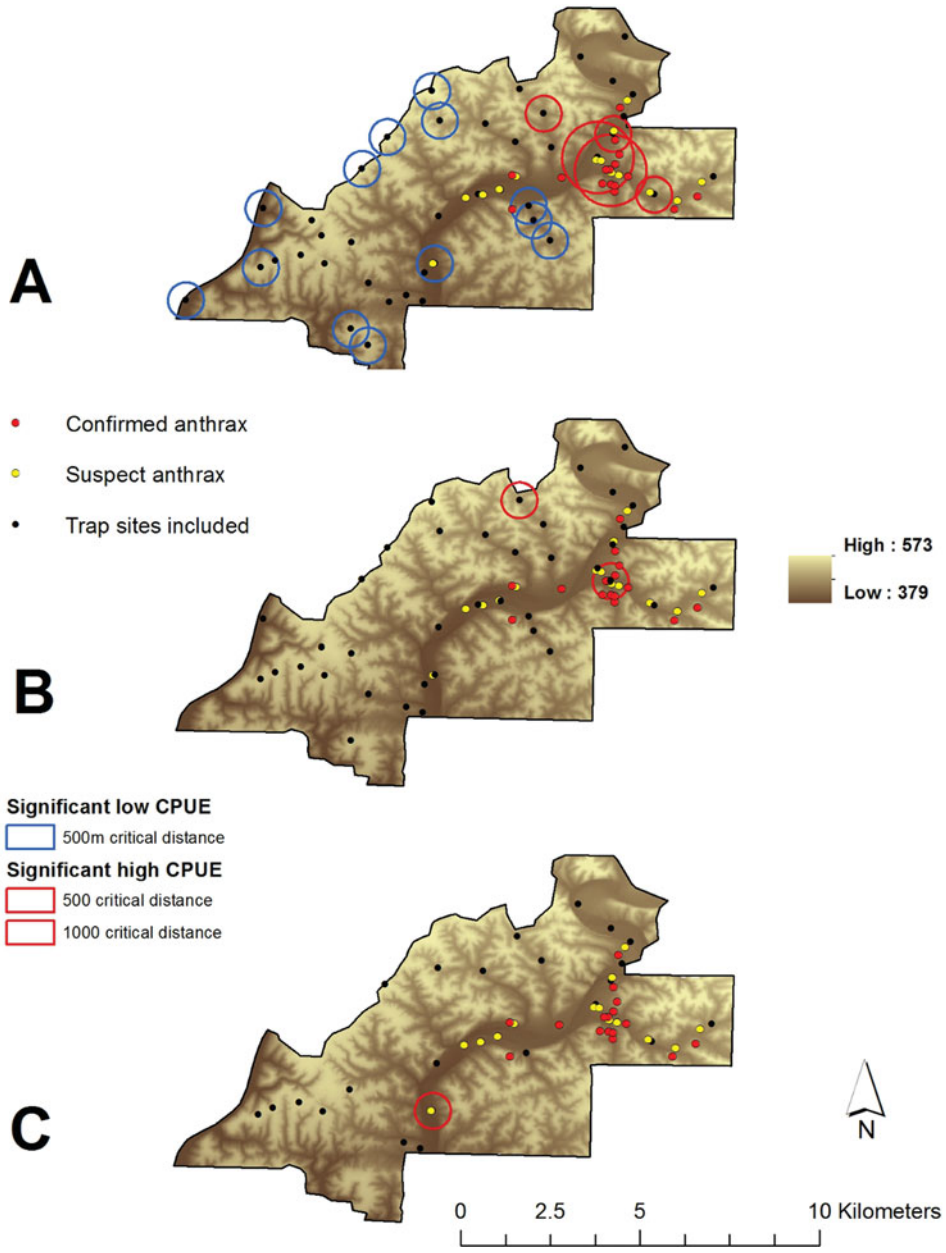

Figure 8. G_i^* cluster locations at 500 m and 1,000 m for (A) Setup 1, (B) Setup 2, and (C) Setup 3. Red circles indicate clusters of high values; blue circles indicate clusters of low values. Carcass sites are indicated by red dots (confirmed cases) and yellow dots (suspect cases). Black dots are all locations where fly traps were run during that setup period. (Color figure available online.)

total biting fly counts, making it implausible to determine feeding persistence. The close proximity between anthrax carcasses (also confirmed by the ANNI clustering results), limited seasonal home ranges of these deer (Blackburn 2006), and localized high deer density, however, suggest interactions between deer and biting flies are highly probable.

The G_i^* values during Setup 1 indicated clusters of low catch values across the western half of the ranch (Figure 8). The northern and western boundaries of the ranch are dominated by a high ridgeline (Figure 5) that is sparsely vegetated (Figure 7B), providing minimal shelter for flies from direct summer sunlight and higher average wind speeds upslope. Figure 7A confirms that wind speeds along this ridgeline were very high. Additionally, deer on this portion of the ranch have to move vertically and horizontally to find patches of vegetation for shade, which might increase the search distance between blood meals for the flies. The combination of these factors makes the western and northern ridgeline less suitable for flies than the eastern river valley.

The spatial distribution of flies on this study ranch indicates a strong relationship between biting fly densities and known sites of anthrax deaths in deer. The scenario described earlier indicates the geography of the ranch

lends itself to greater potential for deer–fly interactions and *B. anthracis* spore survival along the dry river valley and associated habitats. Biting fly numbers and anthrax cases might occur contemporaneously through mutually exclusive ecological mechanisms, but the high overlap between biting fly clusters and anthrax does suggest a relationship warranting future research.

The lack of direct overlap between fly sampling and the timing of the epizootic presents a limitation in this study, as we cannot state with certainty that biting fly numbers remained high during the outbreak period. Our fly catches rates were generally high across the eastern and central sampling sites across the study period, however, including those later sampling efforts that preceded the epizootic. Although traps were not run during the peak of the epizootic, ranch personnel did report high biting fly numbers at carcass sites where workers burned carcasses in response to the outbreak. At the same time, earlier studies on biting flies present evidence of seasonal overlap between biting flies and anthrax, as well as local temporal lags between peak fly numbers and anthrax cases. As stated previously, Olsuf'ev and Lelep (1935) noted a ten-day lag between the peak of the anthrax epizootic and highest fly numbers. During this study, we did see some increase in fly catch rates during the last trapping period just ahead of the deer cases, suggesting a similar lag effect in the Texas enzootic zone. Here we confirm that such increases in fly numbers are limited geographically by local ecological conditions. These areas directly overlap with areas of high anthrax mortality in deer.

Davies (1983) illustrated that tabanid fly numbers corresponded to the summer months in Zimbabwe and were in near perfect agreement with human cutaneous cases associated with a major epidemic from 1978 until 1981. That study suggested that flies might have accounted for the wide geographic extent of disease spread during the epidemic. At a local scale, Olsuf'ev and Lelep (1935) reported that a number of tabanid species were observed trying to feed on horse and cattle carcasses in the first forty-eight hours after death in western Siberia. Despite being unable to engorge on blood during these meals, *B. anthracis* was recovered from mouth parts of individual flies collected on these fresh carcasses. Laboratory results in this same study indicated that viable organism could be recovered up to five days postfeeding on mouth parts and two days in the crop and stomach. They also documented viable organism in the feces. During laboratory and field trials, the authors successfully infected a naïve horse with biting flies. The study did note that the period of exposure for viable organism was short and might limit the likelihood of a tabanid acquiring *B. anthracis* and transmitting, meaning that periods of high host density and fly abundance could increase transmission rates. Likewise, the study noted that anthrax attack rates also coincided with animals, such as horses, that flies prefer to feed on. Horse and cattle cases are often cutaneous (infections at or below the skin), and these sores on animals might encourage feeding by individual flies. In contrast, the study also suggested that the near equal numbers in livestock groups in the southern steppe indicated infection via grazing, where preferential fly meals would not influence outbreak dynamics.

Interestingly, the Olsuf'ev and Lelep (1935) study also suggested that biting flies might pick up bacilli from water and mud near drying puddles. The study observed individual flies defecating fifteen to twenty times per twenty-four hours, suggesting that a single fly could leave upwards of forty deposits during the two days that organism remains viable in the stomach. This could create a new localized focus for infection for other animals in the area. This can be defined as the *case multiplier effect*, where flies can amplify the amount of available bacilli on vegetation immediately around a carcass, increasing the potential for other deer to browse those contaminated plants (Blackburn et al. 2010). During the 2005 deer epizootic, we confirmed blow flies to be contaminated in the same way (Blackburn et al. 2010). Biting flies might then play a role in *case multiplying* through depositing spores on leaves, or host jump, which could partially explain the clustering of deer carcasses during the epizootic. The telemetry data presented here confirm that deer movements are very limited during the anthrax risk period, suggesting that the source of *B. anthracis* infection is likely close to the location of death.

Additionally, earlier studies have also confirmed biting fly activity during livestock epizootics. Mohiyudeen and Krishna Rao (1958) confirmed *B. anthracis* using classical microbiology from biting flies collected from bacteremic animals from a 1957 epizootic in India. In a follow-up study, Krishna Rao and Mohiyudeen (1958) reported on the field conditions and field experiments undertaken during that outbreak. Notably the majority (~90 percent) of livestock cases (domestic cows and buffaloes) were cutaneous form, as would be expected from the injection of bacteria from a fly at the feeding site on the body. Several key field observations were reported: (1) the majority of cases were cutaneous and lesions were associated with preferential feeding sites on the body for tabanids; (2) disease was most severe in

areas of high fly densities; (3) the end of the outbreak coincided with the disappearance of biting flies; (4) three of four flies collected from cutaneous lesions were positive for viable organism sixteen hours after collection, whereas flies randomly collected off the bodies were not (shorter in duration, but in line with the Olsuf'ev and Lelep's [1935] findings); and (5) human cases in the area had cutaneous lesions associated with fly bites and lived with livestock in the home. Although we cannot confirm these observations from the 2005 epizootic, we can confirm that deer carcasses were clustered close together and in habitats that support high biting fly densities. The remaining observations should serve as important hypotheses to test in the Texas enzootic zone in future studies.

Although this study cannot confirm the specific role of biting flies in the deer epizootic, this study supports the finding of these previous studies and confirms clear spatial and ecological relationships between anthrax mortality and fly activity. Likewise, we quantified the numbers and density of flies on the landscape across the risk period and in relation to anthrax mortality. We indicated a positive spatial relationship between confirmed anthrax deaths and biting flies during a major white-tailed deer epizootic in 2005, with high fly numbers throughout the study period overlapping or in proximity to areas of greatest deer anthrax mortality. No diagnostic tests were run to confirm *B. anthracis* in or on biting flies, however. Likewise, individual flies were not identified to species, given a lack of knowledge of which flies in Texas might carry bacilli. The summary of species in Ganeva (2004) suggests mouth parts of several species could transmit *B. anthracis* from blood, and because transmission is mechanical (Turell and Knudson 1987), the majority of fly species are likely capable of moving bacilli. This study provides strong evidence for a role of biting flies in anthrax transmission on the Texas landscape and that this role is underappreciated and underinvestigated. The spatial techniques applied here support a positive relationship between disease cases and fly densities and suggest a direction for future research in this area, specifically to define which Tabanid species present the greatest risk to Texas wildlife and the median spore load of contaminated body parts.

Although there is much to learn about biting fly anthrax transmission, wildlife managers should be alert to increases in biting fly numbers in anthrax-prone areas, using them as a proxy for anthrax risk. In areas of the enzootic zone defined here, where deer are pinned or livestock are present, managers should consider means to control biting flies.

References

Abdulla, P., P. James, S. Sulochana, V. Jayaprakasan, and R. Pillai. 1982. Anthrax in a jaguar (*Panthera onca*). *Journal of Zoo Animal Medicine* 13 (4): 151.

Alexander, K. A., B. Lewis, M. Marathe, S. Eubank, and J. K. Blackburn. 2012. Modeling of wildlife associated zoonoses: Applications and caveats. *Vector-Borne and Zoonotic Diseases* 12 (12): 1005–18.

Aragaon, T. J., M. P. Fay, & D. Wollschlaeger. 2012. EpiTools: R Package for Epidemiological Data and Graphics. http://cran.r-project.org/web/packages/epitools/index.html

Bales, M. E., A. L. Dannenberg, P. S. Brachman, A. F. Kaufmann, P. C. Klatsky, and D. A. Ashford. 2002. Epidemiologic responses to anthrax outbreaks: A review of field investigations, 1950–2001. *Emerging Infectious Diseases* 8 (10): 1163–74.

Bellan, S. E., O. Gimenez, R. Choquet, and W. M. Getz. 2013. A hierarchical distance sampling approach to estimating mortality rates from opportunistic carcass surveillance data. *Methods in Ecology and Evolution* 4 (4): 361–69.

Beyer, H. L. 2010. Geospatial Modeling Environment v. 0.7.2.0. http://www.spatialecology.com

Blackburn, J. 2006. Evaluating the spatial ecology of anthrax in North America: Examining epidemiological components across multiple geographic scales using a GIS-based approach. Doctoral dissertation, Department of Geography and Anthropology, Louisiana State University, Baton Rouge.

Blackburn, J., A. Curtis, T. Hadfield, B. O'Shea, M. Mitchell, and M. Hugh-Jones. 2010. Confirmation of *Bacillus anthracis* from flesh-eating flies collected during a west Texas anthrax season. *Journal of Wildlife Diseases* 46 (3): 918–22.

Blackburn, J., K. McNyset, A. Curtis, and M. Hugh-Jones. 2007. Modeling the geographic distribution of *Bacillus anthracis*, the causative agent of anthrax disease, for the contiguous United States using predictive ecologic niche modeling. *The American Journal of Tropical Medicine and Hygiene* 77 (6): 1103–10.

Broughton, E. 1992. Northwest Territories. Anthrax in bison in Wood Buffalo National Park. *The Canadian Veterinary Journal* 33 (2): 134–5.

Cecere, M. C., G. M. Vazquez-Prokopec, R. E. Gürtler, and U. Kitron. 2004. Spatio-temporal analysis of reinfestation by *Triatoma infestans* (Hemiptera: Reduviidae) following insecticide spraying in a rural community in northwestern Argentina. *The American Journal of Tropical Medicine and Hygiene* 71 (6): 803–10.

Clark, P. J., and F. C. Evans. 1954. Distance to nearest neighbor as a measure of spatial relationships in populations. *Ecology* 35:445–53.

Dale, W., and R. Axtell. 1975. Flight of the salt marsh Tabanidae (Diptera), *Tabanus nigrovittatus, Chrysops atlanticus* and *C. fuliginosus*: Correlation with temperature, light, moisture and wind velocity. *Journal of Medical Entomology* 12 (5): 551–57.

Davies, J. C. A. 1983. Anthrax and flies. *The Zimbabwe Science News* 17 (1): 11–12.

Dragon, D., D. Bader, J. Mitchell, and N. Woollen. 2005. Natural dissemination of *Bacillus anthracis* spores in

northern Canada. *Applied and Environmental Microbiology* 71 (3): 1610–15.
Dragon, D., and R. Rennie. 1995. The ecology of anthrax spores: Tough but not invincible. *The Canadian Veterinary Journal* 36 (5): 295–301.
Elliott, H. 1955. The anthrax epizootic in Louisiana in 1954. *Public Health Reports* 70 (9): 932.
ESRI. 2010. ArcGIS v. 10. Redlands, CA: ESRI.
Fasanella, A., D. Galante, G. Garofolo, and M. H. Jones. 2010. Anthrax undervalued zoonosis. *Veterinary Microbiology* 140 (3–4): 318–31.
Fasanella, A., L. Palazzo, A. Petrella, V. Quaranta, B. Romanelli, and G. Garofolo. 2007. Anthrax in red deer (*Cervus elaphus*), Italy. *Emerging Infectious Diseases* 13 (7): 1118–9.
Foil, L. 1989. Tabanids as vectors of disease agents. *Parasitology Today* 5 (3): 88–96.
Fotheringham, A. S., C. Brunsdon, and M. Charlton. 2000. *Quantitative geography: Perspectives on spatial data analysis*. Thousand Oaks, CA: Sage.
Ganeva, D. 2004. Analysis of the Bulgarian tabanid fauna with regard to its potential for epidemiological involvement. *Bulgarian Journal of Veterinary Medicine* 7:1–8.
Gates, C. C., B. T. Elkin, and D. C. Dragon. 1995. Investigation, control and epizootiology of anthrax in a geographically isolated, free-roaming bison population in northern Canada. *Canadian Journal of Veterinary Research* 59 (4): 256–64.
Getis, A., and J. Aldstadt. 2004. Constructing the spatial weights matrix using a local statistic. *Geographical Analysis* 36 (2): 90–104.
Getis, A., A. C. Morrison, K. Gray, and T. W. Scott. 2003. Characteristics of the spatial pattern of the dengue vector, *Aedes aegypti*, in Iquitos, Peru. *The American Journal of Tropical Medicine and Hygiene* 69 (5): 494–505.
Getis, A., and J. Ord. 1992. The analysis of spatial association by use of distance statistics. *Geographical Analysis* 24 (3): 189–206.
Goodwin, J. T., and B. M. Drees. 1996. The horse and deer flies (Diptera, Tabanidae) of Texas. *Southwestern Entomologist* (Suppl. 20).
Hugh-Jones, M., and J. Blackburn. 2009. The ecology of *Bacillus anthracis*. *Molecular Aspects of Medicine* 30 (6): 356–67.
Hugh-Jones, M., and V. De Vos. 2002. Anthrax and wildlife. *Revue Scientifique et Technique (International Office of Epizootics)* 21 (2): 359–83.
IBM, Inc. 2010. SPSS v. 20. Armonk, NY: IBM, Inc.
Jacquez, G., D. Greiling, H. Durbeck, L. Estberg, E. Do, A. Long, and B. Rommel. 2002. *ClusterSeer user guide 2: Software for identifying disease clusters*. Ann Arbor, MI: TerraSeer Press.
Jeffery, J. A. L., P. A. Ryan, S. A. Lyons, P. T. Thomas, and B. H. Kay. 2002. Spatial distribution of vectors of Ross River virus and Barmah Forest virus on Russell Island, Moreton Bay, Queensland. *Australian Journal of Entomology* 41 (4): 329–38.
Jester, S., and J. Dillard. 2010. Conducting white-tailed deer spotlight surveys in Texas. In *Wildlife management activities and practices: Comprehensive wildlife management planning guidelines for the Post Oak Savannah and Blackland Prairie ecological regions*. Austin, TX: Texas Parks and Wildlife Department. Retrieved from http://www.tpwd.state.tx.us/publications/pwdpubs/media/pwd_bk_w7000_0788.pdf
Kellogg, F. E., A. K. Prestwood, and R. E. Noble. 1970. Anthrax epizootic in white-tailed deer. *Journal of Wildlife Diseases* 6 (4): 226–28.
Kelsall, J., and P. Diggle. 1995. Non-parametric estimation of spatial variation in relative risk. *Statistics in Medicine* 14 (21–22): 2335–42.
Krishna Rao, N. S., and S. Mohiyudeen. 1958. Tabanus flies as transmitters of anthrax—A field experience. *Indian Veterinary Journal* 35:348–53.
Mihok, S. 2002. The development of a multipurpose trap (the Nzi) for tsetse and other biting flies. *Bulletin of Entomological Research* 92 (5): 385–403.
Mohiyudeen, S., and N. S. Krishna Rao. 1958. An epidemic of cutaneous anthrax among bovines in North Kanera District (Mysore State). *Indian Veterinary Journal* 35 (2): 55–63.
Morris, H. 1918. *Blood-sucking insects as transmitters of anthrax or charbon*. Baton Rouge: Agricultural Experiment Station of the Louisiana State University and A&M College.
Nelson, T. A., and B. Boots. 2008. Detecting spatial hot spots in landscape ecology. *Ecography* 31 (5): 556–66.
Olsuf'ev, N. G., and P. P. Lelep. 1935. On the importance of tabanids in the spread of anthrax. In *Parasites, transmetteurs, anim. venimeux. Rec. Trav. 25e Anniv. sci Pavovsky 1909–34*, 145–97. Moscow.
Omernik, J. M. 1995. Ecoregions: A spatial framework for environmental management. In *Biological assessment and criteria: Tools for water resource planning and decision making*, ed. W. S. Davis & T. P. Simon, 49–62. Boca Raton, FL: Lewis Publishers.
Ord, J. K., and A. Getis. 1995. Local spatial autocorrelation statistics: Distributional issues and an application. *Geographical Analysis* 27 (4): 286–306.
Saile, E., and T. Koehler. 2006. *Bacillus anthracis* multiplication, persistence, and genetic exchange in the rhizosphere of grass plants. *Applied and Environmental Microbiology* 72 (5): 3168–74.
Sciarretta, A., M. Girma, G. Tikubet, L. Belayehun, S. Ballo, and J. Baumgärtner. 2005. Development of an adaptive tsetse population management scheme for the Luke community, Ethiopia. *Journal of Medical Entomology* 42 (6): 1006–19.
Sheppard, C., and B. Wilson. 1976. Flight range of Tabanidae in a Louisiana bottomland hardwood forest. *Environmental Entomology* 5 (4): 752–54.
Silverman, B. W. 1986. *Density estimation for statistics and data analysis*. London and New York: Chapman & Hall/CRC.
Smith, K. L., V. DeVos, H. Bryden, L. B. Price, M. E. Hugh-Jones, and P. Keim. 2000. *Bacillus anthracis* diversity in Kruger National Park. *Journal of Clinical Microbiology* 38 (10): 3780–84.
Turell, M. J., and G. B. Knudson. 1987. Mechanical transmission of *Bacillus anthracis* by stable flies (*Stomoxys calcitrans*) and mosquitoes (*Aedes aegypti* and *Aedes taeniorhynchus*). *Infection and Immunity* 55 (8): 1859–61.

Turnbull, P., M. Diekman, J. Killian, W. Versfeld, V. De Vos, L. Arntzen, K. Wolter, P. Bartels, and A. Kotze. 2010. Naturally acquired antibodies to *Bacillus anthracis* protective antigen in vultures of southern Africa. *Onderstepoort Journal of Veterinary Research* 75 (2): 95–102.

Turnbull, P. C. B., Food and Agriculture Organization of the United Nations, World Organization for Animal Health, and World Health Organization. 2008. *Anthrax in humans and animals*. 4th ed. Geneva: World Health Organization.

Van Ness, G. B. 1971. Ecology of anthrax. *Science* 172 (3990): 1303–7.

Van Ness, G., and C. Stein. 1956. Soils of the United States favorable for anthrax. *Journal of the American Veterinary Medical Association* 128 (1): 7–9.

Vazquez-Prokopec, G. M., M. C. Cecere, D. M. Canale, R. E. Gürtler, and U. Kitron. 2005. Spatiotemporal patterns of reinfestation by *Triatoma guasayana* (Hemiptera: Reduviidae) in a rural community of northwestern Argentina. *Journal of Medical Entomology* 42 (4): 571–81.

Ward, M. P., and T. E. Carpenter. 2000. Techniques for analysis of disease clustering in space and in time in veterinary epidemiology. *Preventive Veterinary Medicine* 45 (3): 257–84.

Yanli, L., H. Bisong, G. Jianhua, C. Wuchun, and F. Liqun. 2009. Visual data mining of SARS distribution using self-organization maps. In *Proceedings of the International Conference on Management and Service Science*, 1–4. Wuhan/Beijing: International Conference on Management and Service Science.

Correspondence: Spatial Epidemiology & Ecology Research Laboratory, Department of Geography, University of Florida, Gainesville, FL 32611, e-mail: jkblackburn@ufl.edu (Blackburn); MRI Global, 1470 Treeland Boulevard Southeast, Palm Bay, FL 32909, e-mail: hadecollc@yahoo.com (Hadfield); GIS, Health & Hazards Lab, Department of Geography, Kent State University, 413 McGilvrey Hall, Kent, OH 44242, e-mail: acurti13@kent.edu (Curtis); Department of Environmental Sciences, School of the Coast and Environment, Louisiana State University, Baton Rouge, LA 70802, e-mail: mehj@vetmed.lsu.edu (Hugh-Jones).