

## Potential *Bacillus anthracis* Risk Zones for Male Plains Bison (*Bison bison bison*) in Southwestern Montana

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**ABSTRACT:** A re-emergence of anthrax, a zoonosis caused by the long-lived, spore-forming *Bacillus anthracis*, occurred with a multispecies outbreak in southwestern Montana in 2008. It substantially impacted a managed herd of about 3,500 free-ranging plains bison (*Bison bison bison*) on a large, private ranch southwest of Bozeman, with about 8% mortality and a disproportionate 28% mortality of mature males; a similar high rate occurred in male Rocky Mountain elk (*Cervus canadensis nelson*). Grazing herbivores are particularly at risk for anthrax from ingesting spore-contaminated soil and grasses in persistent environmental reservoirs. We predicted areas of mature male bison habitat preference on the landscape by using GPS collar data and a resource selection function model using environmental covariates. We overlaid preferred areas with ecologic niche, model-based predictions of *B. anthracis* environmental reservoirs to identify areas of high anthrax risk. Overlapping areas were distributed across the ranch and were not confined to pastures associated with the previous outbreak, suggesting that ongoing pasture exclusion alone will not prevent future outbreaks. The data suggested vaccination campaigns should continue for bison, and the results can be used to prioritize carcass surveillance in areas of greatest overlap.

**Key words:** Anthrax, *Bacillus anthracis*, bison, resource selection, southwestern Montana, transmission risk.

Anthrax is a worldwide zoonosis of concern caused by the spore-forming bacterium *Bacillus anthracis* (Hugh-Jones and Blackburn 2009; Fasanella et al. 2010). These spores can remain viable in the soil for extended periods, creating long-lasting pathogen reservoirs on the landscape (Hugh-Jones and Blackburn 2009; Turner et al. 2014). Soil is a primary reservoir for spores, and grazing herbivores can ingest these spores, along with contami-

nated grasses, plants, and roots (Fasanella et al. 2010; Ganz et al. 2014). Herbivores may encounter concentrated spores at carcass sites from previous anthrax deaths, where spores have been found 2 yr or longer after animal death (Lindeque and Turnbull 1994; Turner et al. 2014).

A multispecies outbreak of anthrax occurred on a privately owned ranch in southwestern Montana in summer 2008 (Blackburn et al. 2014; Morris et al. 2016a). Plains bison (*Bison bison bison*) kept as livestock and free-ranging male Rocky Mountain elk (*Cervus canadensis nelson*) were the two species most affected, but at least two white-tailed deer (*Odocoileus virginianus*) were also killed (Morris et al. 2016a). At least 298 bison were lost, with a male skew (about 28% of the male population died versus about 8% of the females; Bagamian et al. 2013). Higher male compared with female disease burden has been observed in several bison anthrax outbreaks (Broughton 1992; Shury et al. 2009; Salb et al. 2014). In this region, anthrax is considered a re-emerging disease, as no cases had been reported in decades (Blackburn et al. 2014).

The study ranch (Fig. 1) occupies 380 km<sup>2</sup> of the Northern Madison Study Area (NMSA; Atwood 2006) and is managed for bison and wildlife. Fences restrict bison to specific pastures, but do not prevent movement of cervids or predators. Previously, Morris et al. (2016a) identified areas where male elk likely overlap with potential anthrax risk zones across the NMSA. Our objective was to model and map male bison resource selection across the ranch during the anthrax risk period

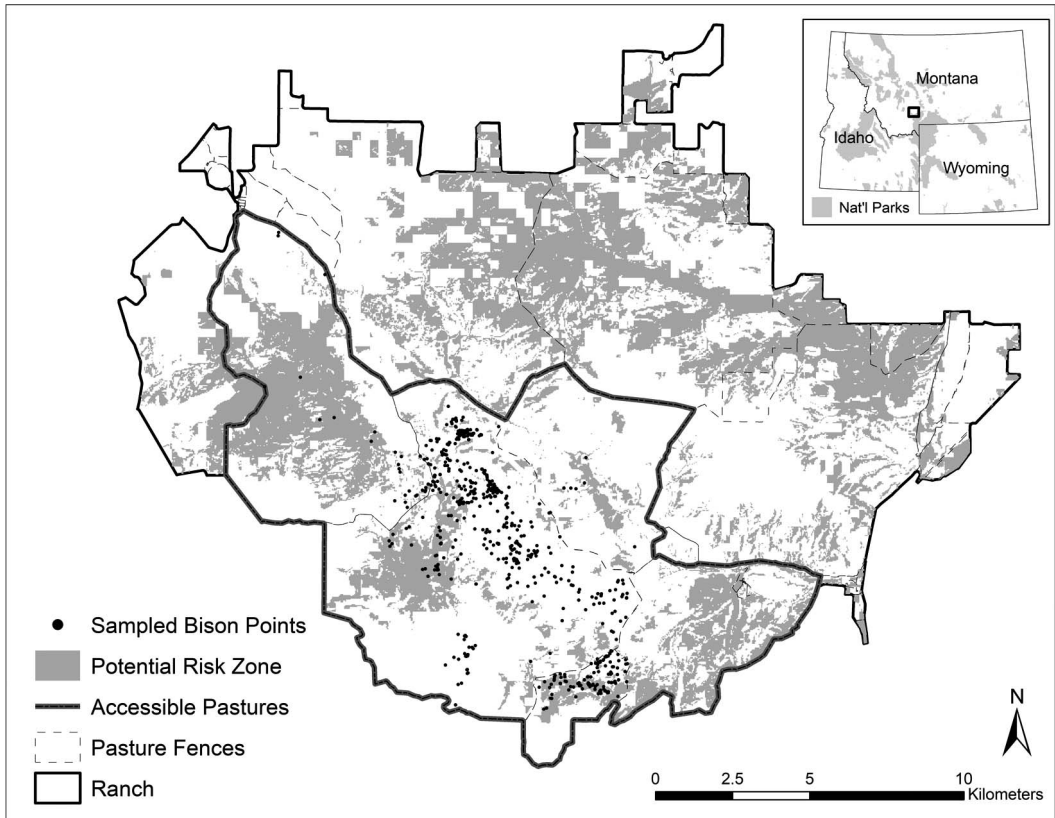


FIGURE 1. Study area with male plains bison (*Bison bison bison*) locations from GPS collars (four random points per animal per day) used to model resource selection. Study ranch boundary and pasture fences, including the pastures accessible to the bison during the study period, are illustrated. The shaded areas represent potential *Bacillus anthracis* risk zones based on an ecologic niche model prediction. Bison resource selection was compared to *B. anthracis* risk zones.

(June–August) and estimate overlap with potential anthrax risk zones defined from the 2008 case locations and two subsequent cases in 2010 (Blackburn et al. 2014).

As a pilot study, adult male bison ( $n=3$ ) were fitted with GPS collars (model GPS3300L, Lotek, Newmarket, Ontario, Canada) recording fixes every 30 min during the 2012 risk period (Table 1). Capture protocols were developed by the ranch wildlife veterinarian (D.L.H.) and approved by the University of Florida Institutional Animal Care and Use Committee (UF IACUC 201207594 to J.K.B.).

Predictions of bison habitat preference were created using a mixed-model logistic regression resource selection function (RSF) with environmental variables as fixed effects

and a random intercept term for individual bison (Manly et al. 2002; Gillies et al. 2006). To remain consistent with Morris et al. (2016a), we used a use-versus-available framework

TABLE 1. Start and end dates for GPS tracking in 2012 and number of subsampled fixes for each collared male plains bison (*Bison bison bison*) during this study. Number of fixes were subsampled to four random points per day per bison (number shown) and used to develop a resource selection function (model to map male bison habitat in southwestern Montana).

Bison	Start	End	No. of fixes
1	1 June	15 August	304
2	1 June	19 July	196
3	1 June	3 July	132

TABLE 2. Environmental covariates used in the resource selection function (RSF) model used to predict plains bison (*Bison bison bison*) habitat use. All covariates were converted to 30-m raster layers to map RSF outputs. Coefficients and SE for fixed effect variables in the final resource selection function model developed to predict bison habitat use during the anthrax risk period in southwestern Montana. All nine environmental variables were significant for estimating bison resource selection ( $P \leq 0.001$ ).

Variable	Description	Data source <sup>a</sup>	Coefficient estimate	SE
Fixed effects intercept	From best model		-1.97	0.204
Aspect	Categorical: southerly (134–224°); not southerly (all other)	Derived from DEM	0.124	0.169
Elev	Elevation	DEM from USGS NED	0.313	0.089
Forest	Categorical: forest versus non-forest	2010 MSDI land cover dataset	-3.357	0.569
NDVI <sup>b</sup>	MODIS <sup>c</sup> derived NDVI, average from 1 Jun–3 August 2012, 250 m	Reverb ECHO NASA; <a href="https://modis.gsfc.nasa.gov/data/">https://modis.gsfc.nasa.gov/data/</a>	0.494	0.074
RD_PRIM	Distance to primary roads	Morris et al. 2016a	0.103	0.067
RD_SEC	Distance to secondary roads	Morris et al. 2016a	-0.744	0.07
RD_TERT	Distance to tertiary roads	Morris et al. 2016a	-0.295	0.058
Slope	Slope	Derived from DEM used for Elev	-0.791	0.085
Stream	Distance to streams	Morris et al. 2016a	0.075	0.062

<sup>a</sup> DEM = digital elevation model; USGS = US Geological Survey; NED = National Elevation Dataset; MSDI = Montana Spatial Data Infrastructure; NASA = National Aeronautics and Space Administration.

<sup>b</sup> Normalized difference vegetation index.

<sup>c</sup> Moderate resolution imaging spectroradiometer.

with available area defined by a minimum convex polygon (100% MCP) around a pool of GPS points (used points) selected from a random draw of four points per day per animal. The MCP was first clipped to pastures available to bison. Five random available points were drawn from within the final MCP for each used point. The mixed model was calculated in R (nlptr package, Johnson 2007; ln\_bobyqa optimizer algorithm, Powell 2009; glmer package, Bates et al. 2015). Accuracy was measured through a fivefold cross validation, and both validation and mapping were performed with equal interval binning (Morris et al. 2016a, b).

We used nine environmental variables (Table 2), those in the elk study (Morris et al. 2016a) plus distance to streams and time-averaged normalized difference vegetation index (NDVI). All variables were resampled to 30-m raster grids to project the model onto the landscape (Morris et al. 2016a). Bison have shown preferences for water, lower elevations, gentler slopes, different landcover types, and roads (Fischer and Gates 2005;

Bruggeman et al. 2007; Fortin et al. 2009; Allred et al. 2011).

We defined potential anthrax risk zones by using a previously published ecologic niche model for this region (Morris et al. 2016a); that study provided three cutoffs for risk. We chose the conservative definition, or most limited estimate, of *B. anthracis* presence and clipped the estimate to ranch boundaries (Fig. 1). In brief, risk zones were defined from a presence-only genetic algorithm by using bison, elk, and white-tailed deer cases from the 2008 outbreak; two bison cases in 2010; and covariates associated with anthrax. The final model set had a total omission of 3.4% or was 96.6% accurate at predicting actual cases (Morris et al. 2016a).

All nine environmental variables were significant for estimating bison resource selection ( $P \leq 0.001$ ; Table 2) and the final model was highly accurate with an average Spearman's rank correlation coefficient from the cross-fold validation of 0.90. The coefficients indicated that mature male bison on the ranch selected areas dominated by non-forest areas,

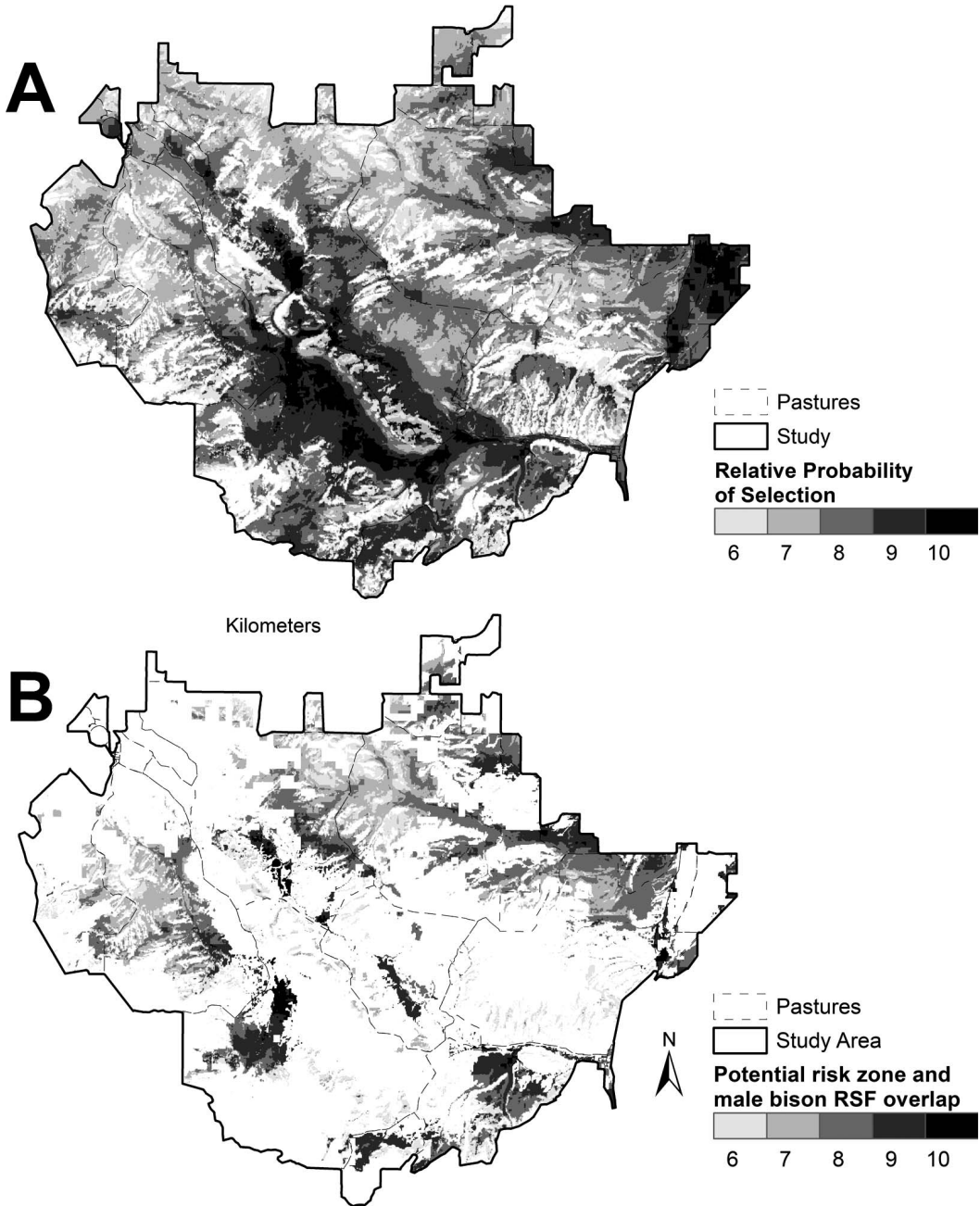


FIGURE 2. Spatial predictions of male plains bison (*Bison bison bison*) habitat preference (top five equal interval bins) from a resource selection function (RSF) model for the study area in southwestern Montana (A) and overlap of RSF-based male bison habitat with potential seasonal anthrax risk zones (B).

gentler slopes, close proximity to secondary and tertiary roads, higher NDVI values, southerly aspects, distances farther from primary roads and streams, and higher

elevations. The random effect for individual bison was statistically significant, indicating variation between the collared males (intercepts of 0.36, 0.05, and  $-0.41$ , respectively).



To illustrate areas to be most likely selected by male bison, we mapped the top five RSF bins (preferred habitat areas) onto the landscape. Preferred areas were in the central and northeastern portions of the ranch (Fig. 2A). The preferred areas overlapped with potential risk zones across 118 km<sup>2</sup>, or about 30% of the area of the ranch. Overlap areas were spread across the ranch and not confined to specific pastures (Fig. 2B). In an effort to prevent disease, bison grazing access has been restricted during the seasonal risk period, and bison remain entirely excluded from pastures that exhibited the highest mortality in 2008 (Morris et al. 2016a). However, our results suggested that there are risk areas available to male bison despite exclusion efforts and that this practice alone may not prevent future outbreaks.

We defined the anthrax risk season as June–August based on the 2008 and 2010 cases. This period overlaps bison rut, wherein sexually mature male bison are competing against each other (Dragon et al. 1999). Rut may alter the bison behaviors (e.g., wallowing) and space preferences (Dragon et al. 1999); stress from rut may increase infection risk and movement away from the herd after rut may make finding dead males difficult. Although our sample size was limited to three individual males, model validation indicated high accuracy for predicting habitat selection during the seasonal anthrax risk period, despite variation in these three individuals. Future work should increase the number of collared bison. Overall, surveillance is advised, particularly in areas of high bison-anthrax risk overlap, including diagnostic testing for any bison deaths in summer. Likewise, disease risk extends beyond current excluded pastures, indicating vaccination should continue. Nearby ranches should also perform testing for unusual livestock deaths in summer and consider vaccination.

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