

The Necrophagous Fly Anthrax Transmission Pathway: Empirical and Genetic Evidence from Wildlife Epizootics

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Abstract

Early studies confirmed *Bacillus anthracis* in emesis and feces of flies under laboratory conditions, but there is little empirical field evidence supporting the roles of flies in anthrax transmission. We collected samples during outbreaks of anthrax affecting livestock and native and exotic wildlife on two ranches in West Texas (2009–2010). Sampling included animal carcasses, maggots, adult flies feeding on or within several meters of carcasses, and leaves from surrounding vegetation. Microbiology and PCR were used to detect *B. anthracis* in the samples. Viable *B. anthracis* and/or PCR-positive results were obtained from all represented sample types. Genetic analysis of *B. anthracis* samples using multilocus variable number tandem repeat analysis (MLVA) confirmed that each ranch represented a distinct genetic lineage. Within each ranch, we detected the same genotype of *B. anthracis* from carcasses, maggots, and adult flies. The results of this study provide evidence supporting a transmission cycle in which blowflies contaminate vegetation near carcasses that may then infect additional browsing animals during anthrax outbreaks in the shrubland environment of West Texas.

Key Words: Anthrax—*Bacillus anthracis*—Disease ecology—Pathogen transmission—Phylogenetics.

Introduction

Bacillus anthracis, the causative agent of anthrax, is a spore-forming Gram-positive bacterium. Anthrax is reported nearly worldwide (Hugh-Jones 1999, Fasanella et al. 2010), although the distribution of naturally occurring disease is limited by ecological factors (Blackburn 2010, Alexander et al. 2012) and *B. anthracis* can be difficult to isolate from the environment. Outbreaks in the United States have been associated with wildlife, particularly white-tailed deer (*Odocoileus virginianus*) in southwest Texas (Blackburn and Goodin 2013), and elk (*Cervus elaphus*), plains bison (*Bison bison bison*), and white-tailed deer in southwestern Montana (Hugh-Jones and Blackburn 2009). Classically, it is hypothesized that herbivore exposure to *B. anthracis* is via ingestion of spores through grazing, browsing, or blood-sucking insect bites, particularly tabanid flies (Alexander et al. 2012). Grazing animals ingest both vegetation and soil simultaneously or ingest large quantities of soil at certain times of year (Turner et al. 2013). White-tailed deer in Texas browse extensively during the summer anthrax

season, so ingestion of spores from grasses and the associated roots and soil is less likely (Blackburn et al. 2010). In Kruger National Park, South Africa, Pienaar (1961) and Braack and de Vos (1990) hypothesized that leaves of preferred browse species were contaminated with *B. anthracis* from fly emesis and feces and subsequently eaten during browsing events by greater kudu (*Tragelaphus strepsiceros*).

This hypothesis was extended to white-tailed deer in West Texas (Hugh-Jones and de Vos 2002) and termed the case multiplier hypothesis, which states such fly-driven contamination could expand the size of an epizootic by increasing contact rates between naïve animals and bacteria deposited on browse through fly droplets. Recently, we showed necrophagous flies of the families Sarcophagidae and Calliphoridae (Diptera) were positive for viable *B. anthracis* when collected from the carcasses of deer that died of anthrax during an epizootic (Blackburn et al. 2010). Experimental fieldwork by Braack and de Vos (1990) demonstrated that flies were most likely to defecate or regurgitate within 1 meter of the carcass where feeding occurred, a behavior that increases the

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likelihood of contaminated droplets being left close to the carcass or origin.

Figure 1 illustrates a necrophagous fly transmission pathway for white-tailed deer. After an animal succumbs to infection and dies, the carcass is colonized by necrophagous flies searching for food and to lay eggs. Maggots and flies may contaminate the outside of the carcass and the soils immediately adjacent to the carcass. At the same time, adult flies leaving the carcass can move bacteria to nearby vegetation in the form of contaminated emesis, deposited during the regurgitation and reingestion of the meal, or in the form of contaminated feces following digestion and defecation. Recent laboratory studies confirmed *Musca domestica* flies defecate viable organisms after short (~2 h) and long feeding bouts (>8 h) on contaminated meat or blood and recovered bacteria counts could be high (Fasanella et al. 2010).

Despite several early studies confirming the presence of *B. anthracis* in the emesis and feces of flies under laboratory conditions (Graham-Smith 1913, Morris 1918), and a modified hypothesis on the role flies play in contaminating vegetation (Braack and De Vos 1990), there is a paucity of empirical evidence from the field to support the roles of flies in anthrax transmission. Much of what we currently know about *B. anthracis* survival is limited to mortality events and associated efforts to isolate culture from carcasses. Here we present evidence that adult flies and maggots carry viable *B. anthracis* in the vicinity of anthrax-positive deer carcasses and contaminate the surrounding vegetation during outbreaks in Texas. Furthermore, we use a high-resolution, 25-marker multiple locus variable number tandem repeat analysis (MLVA) (Lista et al. 2006) to link the bacterial genotype in the insects (both larvae and adults), environment, and animal hosts.

Methods and Materials

Field collection

In this study, we collected bone fragments (nasal turbinates and/or rib bones) and tissues (ears) from dead deer, bison, domestic cows, and the soil surrounding carcasses during anthrax outbreaks in West Texas in 2009 and 2010. We also collected flies on or near the carcasses and larval stages (maggots) from around the carcass or those directly feeding on tissue from the carcass (such as the ear or other body parts collected for diagnostic isolation efforts), maggots in the soil around the carcass (described as the maggot matrix by Pieenaar 1961), and adult flies in proximity, but up to several meters away, from the carcass on surrounding leaf surfaces. Additionally, leaves from shrubs were collected from up to several meters away from the carcass in an effort to detect *B. anthracis* from fly emesis and feces deposited by adults after feeding. All samples were stored in either sterile Whirlpak plastic bags or sterile 50-mL conical screw top plastic tubes, stored on wet ice in the field, and shipped at ambient temperature to MRI Global (Palm Bay, FL) for laboratory diagnostics where they were refrigerated until processed. Ranch locations were recorded with Global Positioning System (GPS) units as longitude/latitude coordinate pairs and mapped. Mortality rates were calculated for each outbreak on the basis of carcass counts and ranch-provided population estimates. Exact binomial confidence intervals (BCIs) were calculated using the epitools package in R following Blackburn and Goodin (2013).

Classical microbiology and PCR were used to detect *Bacillus anthracis* in all samples collected. Briefly, materials were suspended in 100% ethanol for 1 h prior to plating to

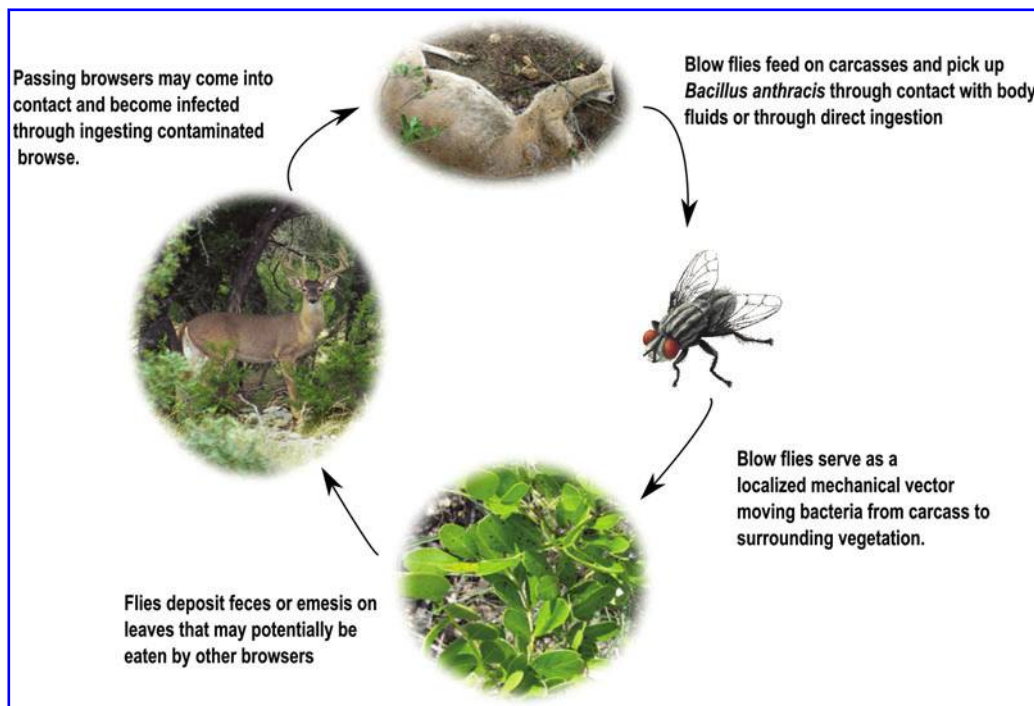


FIG. 1. The necrophagous fly transmission pathway for *Bacillus anthracis*. Blowflies and maggots feed on a dead deer carcass and pick up the bacteria (top). Adult flies leave the carcass body and travel to nearby vegetation carrying the pathogen (right). Bacteria are left behind on leaf surfaces in either emesis or feces (bottom). Nearby browsing animals come into contact with the bacteria leading to a new case (left).

reduce vegetative populations of bacteria in place of heat shocking. Bones were broken into fragments and tissues were cut into small pieces before suspension in ethanol (~1–2 grams of tissue to 9 mL of ethanol). Samples were concentrated by centrifugation, and the alcohol was replaced with molecular-grade water. The pellet was suspended in 1 mL of water, vortexed for 30 s, and then streaked on sheep blood agar and ACTSBA medium 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 and MLVA-25 genotyping.

Leaf preparation

Leaves were diced into small pieces using sterile scissors. The small pieces with presumptive droplets of fly vomit and feces were placed into 1.5-mL tubes and were further macerated using a tissue grinder. After macerating for 1–2 min, 500 μ L of sterile phosphate-buffered saline was added to the tubes and the leaves were macerated for an additional minute. The tubes were centrifuged at 500 rpm for 2 min to sediment the leaf pieces. The supernatant was collected into a sterile 1.5-mL tube. A portion of the original suspension (post-maceration) was submitted for DNA extraction to test for presence of *B. anthracis*.

The remaining supernatant was centrifuged at 12,000 rpm in a microcentrifuge for 5 min to pellet spores and other organisms. The supernatant was removed and the cell pellet was suspended in 100% ethanol for 1 h. The suspension was vortexed to mix the spores, and 100 μ L of suspension was plated on 5% sheep blood agar. The plates were incubated overnight at 37°C. Plates were examined the next day for colonies resembling *B. anthracis*. Suspect colonies were harvested into sterile water and were subsequently subcultured for phage sensitivity and PCR analysis. A gamma phage suspension (10 μ L) was placed on the inoculum and incubated overnight. The following day, the plates were inspected for plaques due to the gamma phage on the lawn of bacterial cells.

DNA extraction

The cells/spores were pelleted by centrifugation and subsequently were suspended in Tris-EDTA (TE) buffer. The cells were lysed using a bead-beating step followed by DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD) following the Gram-positive bacterial protocol. DNA was suspended in sterile molecular-grade water for PCR.

Polymerase chain reaction

PCR for lethal factor (pX01 plasmid), capsule (pX02 marker), and a chromosomal marker was used to confirm the identity of *B. anthracis*. Commercially prepared BioRad PCR master mix was used for the amplification. Amplification conditions were 95°C for 2 min followed by 45 cycles of 95°C for 15 s and 60°C for 1 min. Each assay was performed individually in a BioRad iCycler using the primer sequences in Table 1.

MLVA-25 genotyping

MLVA-25 genotyping was performed as described by Lista et al. (2006), with minor changes in PCR chemistry and

TABLE 1. GENE TARGETS AND PRIMERS FOR PCR TO DETECT *BACILLUS ANTHRACIS*

Gene target	Forward primer	Reverse primer	Probe
Lethal factor	CACTATCAACACTGGAGCGATTCT	AATTATGTCATCTTCTTTGGCTCAA	6FAM- AGCTGCAGATTCC-BHQ
<i>capB</i>	TAAGCCCTCGTCTTCGTAAATG	GTTCCCAAATACGTAATGTTGATGAG	6FAM- TTGCAGCGAATGAT-BHQ
BA-1 chromosome	GTACATCTTCTAGCTGTGCAA	ACGTAGGAAGACCGTTGATTA	6FAM- CGTTGTTGTTGATTG-BHQ

adaptations in primer labeling to allow five-dye fluorescent genotyping using ABI chemistries and platforms. Multiplex PCR products were diluted 1:25 in molecular-grade water, and 0.5 µL of the diluted multiplexes were mixed with 9.5 µL of a formamide/LIZ 1200 (Applied Biosystems, Foster City, CA) size standard mixture and denatured. Fragment sizing for MLVA-25 was performed on an ABI 3100 (Applied Biosystems), and VNTR sizes were determined using GeneMapper™ software (Applied Biosystems).

We examined genetic relationships among samples in the context of global representatives from Lista et al. (2006) using unweighted pair group method with arithmetic averages (UPGMA) cluster analysis. Matrix distances were calculated in PAUP 4.0 (Sinauer Associates, Inc., Sunderland, MA) and imported into MEGA 5.2 (Tamura et al. 2007) to build a phylogenetic tree based on the MLVA-25 genotypes.

Major *B. anthracis* lineages were identified based on Keim et al. (2000) nomenclature as interpreted by Lista et al. (2006).

Results

Field sampling

Animal tissues, bone fragments, flies, and maggots were collected from two outbreaks on geographically separated ranches (~55 km apart) during the summer months of 2010 (Fig. 2). Both ranches are situated on the Edwards Plateau, an area with a history of anthrax in wildlife and livestock (Blackburn and Goodin 2013). First, samples from a plains bison cow and eight white-tailed deer were collected from a large epizootic on a ~2428 hectare (~6000 acre) wildlife ranch managed for hunting ~75 km north of Del Rio, Texas

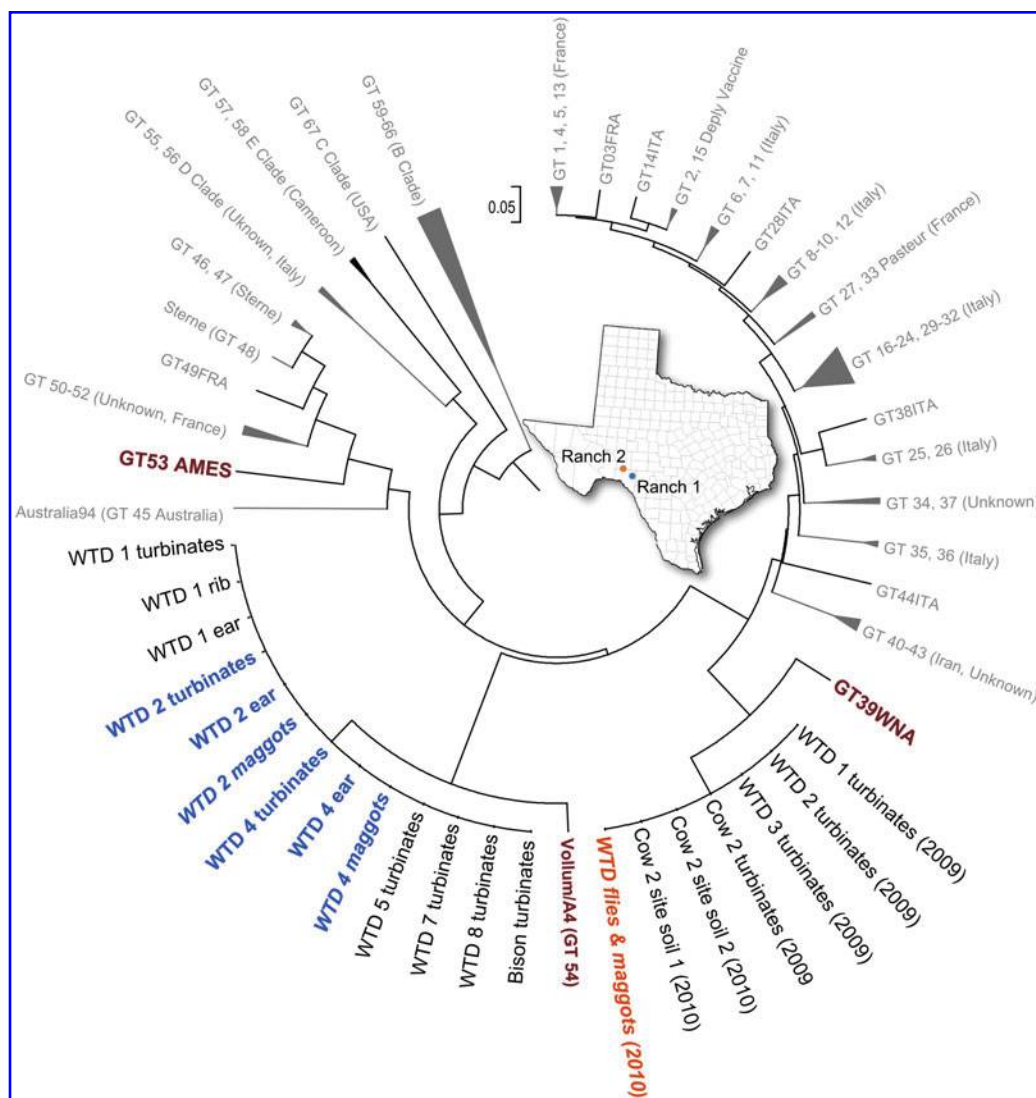


FIG. 2. MLVA-25 defined phylogenetic relationships of *B. anthracis* recovered from necrophagous flies and maggots and deer, bison, cow, and soil samples during outbreaks in 2009 and 2010 in West Texas. Bold text represents isolates from animal samples associated with either flies or maggots. Bold, italicized text represents isolates from flies or maggots. Red text identifies the dominant lineages associated with outbreaks in Texas. Gray samples are those reported by Lista et al. (2006). The map inset illustrates the location of each ranch. Map symbol colors correspond to text color in the tree for each ranch: blue=ranch 1, orange=ranch 2.

(ranch 1). All ranch 1 samples were collected on July 19, just over a month after the cases began and midway through the documented cases. All cases sampled on this ranch had an estimated date of death of at least 10 days prior to collecting samples, many closer to 21 days, on the basis of ranch personnel estimates of when carcasses were discovered and body condition at the time of sampling.

Flies were collected from the deer and bison and on leaves surrounding carcasses. The index case of the outbreak was a white-tailed buck (male) found on July 16, 2010 and the outbreak lasted through mid-August, with at least 50 dead animals, including bison ($n=3$), white-tailed deer ($n=46$), and fallow deer (*Dama dama*), an exotic Eurasian species introduced and often managed in Texas ($n=1$). The three bison deaths accounted for all bison on the ranch. The total white-tailed deer population was estimated at ~ 900 , for an estimated mortality rate of 5.11% (95% BCI = 3.77–6.76). In response to the outbreak, ranch personnel rounded up animal bones and hides from dead animals and burned them in a single burn pile. Additionally, on the basis of results of this study, each carcass site and ~ 10 meters of vegetation surrounding the carcass were sprayed with a 10% bleach solution from backpack sprayers and water troughs were bleached. Viable *B. anthracis* was recovered from six of eight white-tailed deer and one bison cow (Fig. 2). Flies and maggots were collected from one bison site and four white-tailed deer and culture positive maggots were recovered from the ear collected from deer #4 and the soil around deer #2 (Fig. 2). Additionally, we had PCR confirmation of *B. anthracis* from an adult fly collected on a leaf several meters from the carcass. In total, we recovered bacterial isolates from six deer (6/8 sampled 75% isolate recovery), one bison (1/1 sampled; an additional bison was confirmed positive by the Texas Veterinary Medicine Diagnostic Laboratory), and two maggot pools (2/4; 50% recovery). We did not recover viable organism from leaves based on culturing; however, PCR results confirmed *B. anthracis* was present in four out of five leaves (80%) with fly droplets. DNA samples from the leaves were exhausted by molecular diagnostic PCR and, therefore, not available for retrospective MLVA-25 genotyping.

Samples were also collected on July 19, 2010, from a single white-tailed deer doe found dead on a ranch approximately 45 km northwest of ranch 1 and 40 km south of Ozona, Texas (ranch 2). The site visit to the ranch was a 1-year follow up to a July, 2009, outbreak. On October 9, 2009, we collected diagnostic samples from white-tailed deer and cows associated with the July, 2009, outbreak. A total of 12 cows died during the 2009 outbreak from a population of ~ 32 for a mortality rate of $\sim 37.5\%$ (95% BCI 21.1–56.3). An estimated 30 deer died out of an estimated population of 300 for a mortality rate of $\sim 10\%$ (95% BCI 6.85–13.97). However, results from a helicopter survey for deer during the 2009–2010 winter period suggested much greater losses, with fewer than 10 deer sighted. No decontamination efforts were implemented by ranch personnel during the 2009 outbreak. We returned in July, 2010, to resample soil and water troughs near the epicenter of the 2009 event. In this current study, we isolated viable *B. anthracis* from one fly/maggot pool (1/2; 50% recovery) collected in proximity to a freshly dead (still warm to the touch) white-tailed doe found dead on the property boundary of the ranch during the 2010 visit.

For comparison of samples in each year, we report the MLVA-25 genotypes of *B. anthracis* isolated from three white-tailed deer and one cow sampled in 2009. Additionally, during the 2010 site visit, we recovered and genotyped viable organisms from the soil associated with the carcass site of cow #2 from 2009.

All cultures genotyped from maggots, deer, and bison on ranch 1 were identical on MLVA-25 and were most closely related to the Vollum/A4 lineage. All viable organisms recovered from ranch 2 were identical on MLVA-25 for samples collected in either 2009 or 2010, including the soil isolate recovered a year later, and were most closely related to the A1.a/Western North American lineage. All genetic relationships are illustrated on the phylogenetic tree in Figure 2. Repeat structure at each allele in the MLVA-25 is presented in Table 2.

Discussion

The results of this study provide evidence supporting an animal-to-plant transmission cycle through blowfly vectors during anthrax outbreaks in the shrubland environment of West Texas. Flies and maggots were collected from animals ranging from recently to long dead during a very hot, dry summer period. Despite this, these samples and the animal hosts and insects were positive for viable *B. anthracis* and possessed the genetic markers for virulence. This further substantiates previous sampling on a study ranch west of ranch 1 and south of ranch 2, where viable organisms were recovered from adult flies collected at a deer carcass (Blackburn et al. 2010). Of environmental samples collected at outbreaks, this was the first time we collected leaves with fly spots, necessitating development of new sample-processing protocols for culture and molecular detection. Although we did not recover viable organism from leaves, we had positive PCR confirmations of *B. anthracis* demonstrating presence of the organism on leaves. Lack of culture may be a limitation in our protocol for successfully recovering viable vegetative *B. anthracis* cells from leaf matter. Additionally, leaf collection was opportunistic, limited to few leaves per plant, at a small proportion of the total carcass sites visited. It is also important to recognize that environmental recovery of viable *B. anthracis* necessitates aggressive methods to reduce background organisms, which in turn may limit the sensitivity to culture *B. anthracis* from environmental samples.

Alternatively, our results suggest there is a limited period of survivability away from the host. Additionally, flies found 3–5 meters away from the nearest carcass were carrying viable bacteria, indicating the zone of contamination around the carcass exceeds several meters in diameter. Although we did not make efforts to measure the spatial extent of contamination, we did note fly droplets could be found greater than 2 meters above the ground and in excess of 5 meters from the carcass. Future efforts should determine the proportion of these droplets positive for *B. anthracis*, the bacterial loads present, and the period of time *B. anthracis* cells remain viable.

Here we demonstrate *B. anthracis*-positive samples at each step of the local transmission pathway between animal, flies, and vegetation (positive on either culture or PCR for the pathogen); our study suggests the pathway is spatiotemporally constrained. For example, the lack of viable cells

TABLE 2. MLVA-25 REPEATS FOR ISOLATES RECOVERED FROM NECROPHAGOUS FLIES, MAGGOTS, ANIMAL CARCASSES, AND SOIL FROM OUTBREAKS IN WEST TEXAS 2009 AND 2010

Host species	Sample description (matches phylogenetic tree)	Genotype group (MLVA)	MLVA-25 repeats ^a																								
			01-vrA	02-vrB1	03-vrB2	04-vrC1	05-vrC2	06-CG3	07-pXO1	08-pXO2	09-Bams1	10-Bams3	11-Bams5	12-Bams13	13-Bams15	14-Bams21	15-Bams22	16-Bams23	17-Bams24	18-Bams25	19-Bams28	20-Bams30	21-Bams31	22-Bams34	23-Bams44	24-Bams51	25-Bams53
White-tailed deer	WTD 1 turbinates (2009)	A1.a WNA	9	16	7	57	21	1	8	8	13	29	7	30	49	10	16	11	10	13	14	72	64	9	12	9	8
White-tailed deer	WTD 2 turbinates (2009)	A1.a WNA	9	16	7	57	21	1	8	8	13	29	7	30	49	10	16	11	10	13	14	-1	64	9	12	9	8
White-tailed deer	WTD 3 turbinates (2009)	A1.a WNA	9	16	7	57	21	1	8	8	13	29	7	30	49	10	16	11	10	13	14	72	64	9	12	9	8
Domestic cow	Cow 2 turbinates (2009)	A1.a WNA	9	16	7	57	21	1	8	8	13	29	7	30	49	10	16	11	10	13	14	72	64	9	12	9	8
Soil	Soil sample 1 from cow 2 recovered 1 year later	A1.a WNA	9	16	7	57	21	1	8	8	13	29	7	30	49	10	16	11	10	13	14	72	64	9	12	9	8
Soil	Soil sample 2 from cow 2 recovered 1 year later	A1.a WNA	9	16	7	57	21	1	8	8	13	29	7	30	49	10	16	11	10	13	14	72	64	9	12	9	8
Flies and maggots	Flies and maggots recovered from WTD carcass (2010)	A1.a WNA	9	16	7	57	21	1	8	8	13	29	7	30	49	10	16	11	10	13	14	72	64	9	12	9	8
Plains bison	Bison turbinates	A4/Vollum	9	16	6	48	21	2	10	9	16	30	7	20	44	10	16	11	11	13	14	71	64	9	6	9	8
White-tailed deer	WTD 2 ear (2010)	A4/Vollum	9	16	6	48	21	2	10	9	16	30	7	20	44	10	16	11	11	13	14	71	64	9	6	9	8
White-tailed deer	WTD 1 ear (2010)	A4/Vollum	9	16	6	48	21	2	10	9	16	30	7	20	44	10	16	11	11	13	14	71	64	9	6	9	8
White-tailed deer	WTD 1 rib bone (2010)	A4/Vollum	9	16	6	48	21	2	10	9	16	30	7	20	44	10	16	11	11	13	14	71	64	9	6	9	8
White-tailed deer	WTD 4 ear (2010)	A4/Vollum	9	16	6	48	21	2	10	9	16	30	7	20	44	10	16	11	11	13	14	71	64	9	6	9	8
Maggots	WTD 2 maggots collected from the carcass (2010)	A4/Vollum	9	16	6	48	21	2	10	9	16	30	7	20	44	10	16	11	11	13	14	71	64	9	6	9	8
Maggots	Maggots collected from WTD 4 ear (2010)	A4/Vollum	9	16	6	48	21	2	10	9	16	30	7	20	44	10	16	11	11	13	14	71	64	9	6	9	8
White-tailed deer	WTD 1 turbinates (2010)	A4/Vollum	9	16	6	48	21	2	-1	-1	16	30	7	20	44	10	-1	-1	11	13	14	-1	64	9	6	-1	8
White-tailed deer	WTD 2 turbinates (2010)	A4/Vollum	9	16	6	48	21	2	10	9	16	30	7	20	44	10	16	11	11	13	14	71	64	9	6	9	8
White-tailed deer	WTD 4 turbinates (2010)	A4/Vollum	9	16	6	48	21	2	10	9	16	30	7	20	44	10	16	11	11	13	14	71	64	9	6	9	8
White-tailed deer	WTD 5 turbinates (2010)	A4/Vollum	9	16	6	48	21	2	10	9	16	30	7	20	44	10	16	11	11	13	14	71	64	9	6	9	8
White-tailed deer	WTD 7 turbinates (2010)	A4/Vollum	9	16	6	48	21	2	10	9	16	30	7	20	44	10	16	11	11	13	14	71	64	9	6	9	8
White-tailed deer	WTD 8 turbinates (2010)	A4/Vollum	9	16	6	48	21	2	10	9	16	30	7	20	44	10	16	11	11	13	14	71	64	9	6	9	8

^aMissing alleles are coded as -1. MLVA, multiple locus variable number tandem repeat analysis.

detected on leaves may be a result of the relatively long periods of time between when the animal died, flies deposited the sample, and sample collection. In at least two cases, this period was >21 days from host death to sample collection. This suggests *B. anthracis* has a finite period of survival in fly droplets on browse. Spore persistence is intrinsically limited by environmental conditions such as ultraviolet light, high temperatures, or competition from other organisms (Hugh-Jones and Blackburn 2009), and many of these conditions are likely to be met on leaf surfaces during hot summer periods at this latitude. This is also consistent with the case multiplier hypothesis, suggesting fly-mediated transmission is likely limited to the time period during the outbreak (Alexander et al. 2012) and is not associated with the long-term persistence documented in soils. We note anecdotally that rain events end acute outbreaks in this area, potentially serving as a physical removal process washing *B. anthracis* off of leaves.

The genotyping results confirm a single genotype was present in the animal hosts and invertebrate scavengers during an outbreak and within the transmission pathway. This was true for both ranches, although each ranch represented divergent genetic lineages of *B. anthracis*. The molecular diagnostic and genotyping indicate *B. anthracis* was being carried away from the carcass into the environment, providing specific evidence that flies move the bacteria from the host onto surrounding plants, which serve as browse for other hosts. One of the positive PCR leaf samples came from Texas Persimmon, *Diospyros texana*, a major component of the deer diet in West and South Texas (Fulbright and Ortega-s 2006). Both of these ranches are situated on the Edwards Plateau of West Texas, where Texas Persimmon can account for up to 10% of an individual deer's diet during the summer months (Fulbright and Ortega-s 2006).

On the basis of these data for an epizootic to be initiated in Texas shrublands, we posit the initial herbivore case is infected through ingestion of contaminated soil or grass, with subsequent cases infected via ingestion of contaminated graze or browse, by licking infected carcasses, and/or from bites from hematophagous flies (Blackburn 2010, Blackburn et al. 2010). Our data suggest necrophagous flies disseminate the pathogen out to surrounding vegetation, making it biologically available for subsequent infection in browsing hosts. Our understanding of the necrophagous fly transmission pathway and its significance would benefit from intensive field sampling providing quantitative assessment of *B. anthracis* contamination around the carcass through space and time.

Whereas carcass burning is currently the primary method for decontamination (Turnbull et al. 2008), our results suggest outbreak control efforts should include decontamination of vegetation immediately surrounding the carcass. When burning is feasible, we suggest using the surrounding vegetation as fuel, thereby destroying fly droplets and potentially contaminated browse as well as the carcass. If burning is not feasible, managers should decontaminate using a bleach solution sprayed on the ground and vegetation surrounding the carcass.

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Author Disclosure Statement

No competing financial interests exist.

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