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Authors: Barandongo, Zoe R., Mfune, John K. E., and Turner, Wendy C.

Source: Journal of Wildlife Diseases, 54(1) : 34-44

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/2017-04-069

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DOI: 10.7589/2017-04-069

Journal of Wildlife Diseases, 54(1), 2018, pp. 34–44
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DUST-BATHING BEHAVIORS OF AFRICAN HERBIVORES AND THE POTENTIAL RISK OF INHALATIONAL ANTHRAX

Zoe R. Barandongo,[1,2] John K. E. Mfune,[1] and Wendy C. Turner[2,3]

[1] Department of Biological Sciences, Faculty of Science, University of Namibia, Private Bag 13301, Windhoek, Namibia
[2] Department of Biological Sciences, State University of New York, University at Albany, 1400 Washington Avenue, Albany, New York 12222, USA
[3] Corresponding author (email: wcturner@albany.edu)

ABSTRACT: Anthrax in herbivorous wildlife and livestock is generally assumed to be transmitted via ingestion or inhalation of *Bacillus anthracis* spores. Although recent studies have highlighted the importance of the ingestion route for anthrax transmission, little is known about the inhalational route in natural systems. Dust bathing could aerosolize soilborne pathogens such as *B. anthracis*, exposing dust-bathing individuals to inhalational infections. We investigated the potential role of dust bathing in the transmission of inhalational anthrax to herbivorous wildlife in Etosha National Park, Namibia, an area with endemic seasonal anthrax outbreaks. We 1) cultured soils from dust-bathing sites for the presence and concentration of *B. anthracis* spores, 2) monitored anthrax carcass sites, the locations with the highest *B. anthracis* concentrations, for evidence of dust bathing, including a site where a zebra died of anthrax on a large dust bath, and 3) characterized the ecology and seasonality of dust bathing in plains zebra (*Equus quagga*), blue wildebeest (*Connochaetes taurinus*), and African savanna elephant (*Loxodonta africana*) using a combination of motion-sensing camera traps and direct observations. Only two out of 83 dust-bath soils were positive for *B. anthracis*, both with low spore concentrations ($\leq$20 colony-forming units per gram). We also detected no evidence of dust baths occurring at anthrax carcass sites, perhaps due to carcass-induced changes in soil composition that may deter dust bathing. Finally, despite observing some seasonal variation in dust bathing, preliminary evidence suggests that the seasonality of dust bathing and anthrax mortalities are not correlated. Thus, although dust bathing creates a dramatic cloud of aerosolized soil around an individual, our microbiologic, ecologic, and behavioral results in concert demonstrate that dust bathing is highly unlikely to transmit inhalational anthrax infections.

Key words: *Bacillus anthracis*, *Connochaetes taurinus*, dust bathing, *Equus quagga*, Etosha National Park, inhalational anthrax, *Loxodonta africana*.

INTRODUCTION

Bacillus anthracis, the causative agent of anthrax, is a bacterium that forms spores capable of surviving in the environment for decades (Manchee et al. 1981). Natural anthrax infections can manifest in three forms, based on the route of infection: gastrointestinal, pulmonary, or cutaneous (WHO 2008). In herbivores, anthrax is primarily transmitted through ingestion of vegetation or soil contaminated with *B. anthracis* spores (Nicholson 2002; Turner et al. 2014, 2016). Inhalation is considered a possible route of transmission for herbivorous wildlife and livestock, with dust bathing postulated as a behavior facilitating pathogen exposure (Turnbull et al. 1998; Dragon et al. 1999; Mackintosh et al. 2002; Turner et al. 2013). There has been little investigation of natural transmission of inhalational anthrax in wildlife. A study of windborne transmission from carcass sites found little risk of inhalational exposure (Turnbull et al. 1998). Dust bathing is a more active form of soil disturbance than wind, and, if *B. anthracis* is present in these soils, dust-bathing animals could be exposed to aerosolized spores.

Dust bathing has several potential benefits and costs for animals that engage in this behavior. Benefits include cleansing the skin, feathers, or fur, removing ectoparasites, and aiding thermoregulation (Joubert 1972; van Liere and Bokma 1987; Rees 2002), while costs include overcrowding (Campbell et al. 2016), increased ectoparasite transmission among aggregated animals (Arzamendia et al. 2012), and, as evaluated in this study, possible exposure to soilborne pathogens. Although widely documented in wild and

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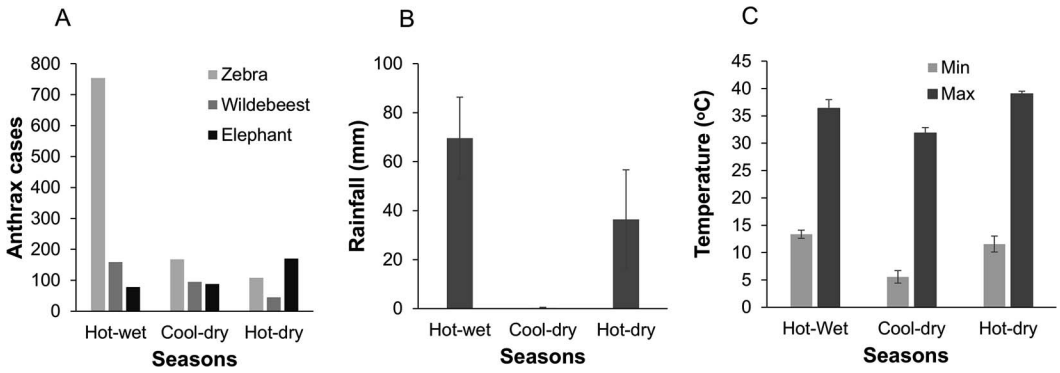


FIGURE 1. Seasonal variation in anthrax mortalities, rainfall, and temperature in Etosha National Park (ENP), Namibia. (A) Anthrax mortalities recorded for the target species in ENP 1975–2014. (B) Average seasonal rainfall recorded in ENP, 2012–14. (C) Average mean monthly temperature minimums and maximums recorded just south of ENP 2012–14. Temperature data were obtained from Namibia Weather Network (2014); rainfall and mortality data were obtained from the Etosha Ecological Institute. The seasons were grouped as follows: hot-wet (January–April), cool-dry (May–August), and hot-dry (September–December).

domestic avian species (Vestergaard 1982; Li et al. 2011), dust bathing has rarely been a focus of study in mammals. Dust bathing in mammals has been noted in reference to landscape heterogeneity or habitat selection (Polley and Wallace 1986; Stapp and Lindquist 2007; Coppedge et al. 2009) and activity patterns (Joubert 1972).

We studied an unmanaged wildlife anthrax system in Etosha National Park (ENP), Namibia. We documented the ecology and seasonality of dust bathing for our target species, as these patterns have not been previously reported. We focused on three herbivore species known to dust bathe that also regularly die of anthrax in ENP: plains zebra (*Equus quagga*), blue wildebeest (*Connochaetes taurinus*), and African savanna elephant (*Loxodonta africana*). We tested soils from dust-bath sites for *B. anthracis*, and monitored anthrax carcass sites for evidence of dust bathing. We assessed our results to determine if dust bathing represents a credible pathway for inhalational transmission of anthrax in an unmanaged wildlife system.

MATERIALS AND METHODS

Study area

Our study was done in ENP, a large, protected semiarid savanna habitat in north-central Namibia, comprising approximately 22,915 km^2. Three seasons are recognized in ENP: the hot-wet season (January–April), the cool-dry season (May–August), and the hot-dry season (September–December). Seasonal differences in recorded anthrax mortalities, rainfall, and temperature are shown in Figure 1. Perennial water in ENP only occurs at point sources, including natural springs and boreholes.

In Namibia, anthrax is considered an endemic disease, and cases are recorded annually (Beyer et al. 2012). Although cases in ENP occur throughout the year, anthrax mortalities peak toward the end of the wet season (March–April) in plains zebra, blue wildebeest, and springbok (*Antidorcas marsupialis*), and a smaller peak in anthrax cases is recorded toward the end of the dry season (October–November) in African elephants (Lindeque and Turnbull 1994; Beyer et al. 2012; Turner et al. 2013). Globally, anthrax outbreaks tend to be seasonally triggered by factors such as extreme rain or drought, with the seasonality varying among systems (Hampson et al. 2011; Blackburn and Goodin 2013).

Screening of dust-bath soils for *B. anthracis*

We opportunistically sampled dust-bath soils throughout ENP from March 2013 to July 2014 and screened them for *B. anthracis*. Sampling sites were those where individuals were observed dust bathing, or from identifiable dust baths. Active dust-bathing sites are readily detectable, because they are devoid of vegetation, have loose soil (either fine powdery or sandy soils, depending on soil composition), and show evidence of recent animal presence (Fig. 2). Surface soil (1–2 cm in depth) was collected from dust-bathing sites. For zebra and blue wildebeest sites, soil was collected

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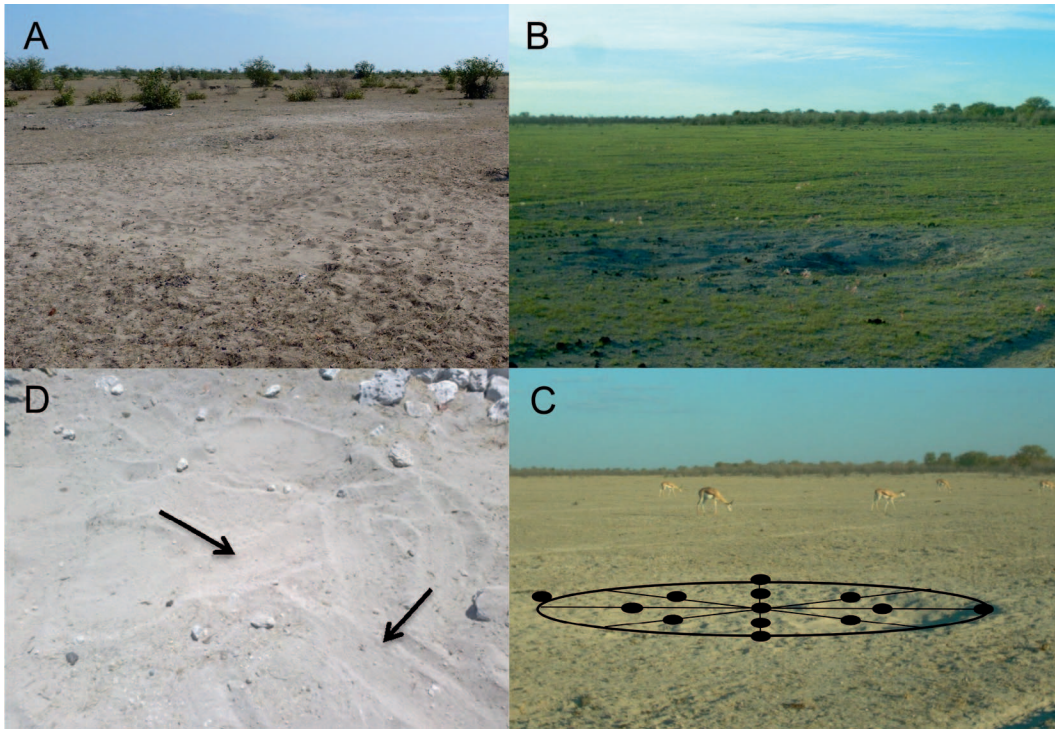


FIGURE 2. Herbivore dust-bathing sites in Etosha National Park, Namibia. (A) Plains zebra (*Equus quagga*) dust-bathing site in the dry season. (B) Blue wildebeest (*Connochaetes taurinus*) dust-bathing site in the wet season and (C) the same dust-bathing site during the dry season, showing the sampling design used for collecting soil samples at zebra and wildebeest dust baths. The lines are transects from the center of the dust bath, and dots indicate the areas from which soil was collected. (D) African elephant (*Loxodonta africana*) dust-bath site. Sampling of elephant dust baths occurred along the ground markings left by the elephant's trunk, indicated with arrows. Photo credits: (A) Nina Pries, (B–D) Z.R.B.

by scooping soil with a sterile spoon starting in the center and moving out toward the marginal ends of the dust bath in a cross section and diagonally (Fig. 2C). Elephant dust baths were sampled by scooping soil along the trunk markings left in the soil (Fig. 2D). Dust-bath soils were cultured on polymyxin-lysozyme-ethylenediaminetetraacetic acid thallous acetate agar to detect the presence and abundance of *B. anthracis* using standard soil protocols (WHO 2008). Whenever culturing, we included soil from an anthrax carcass site as a positive control. We confirmed in subculture each colony identified as *B. anthracis* from dust baths, by testing for penicillin sensitivity and lysis by gamma phage (WHO 2008). *Bacillus anthracis* concentrations were recorded in colony-forming units (CFU) per gram of soil.

Monitoring anthrax carcass sites for dust-bathing activity

Since anthrax carcass sites harbor the highest concentrations of *B. anthracis* found in the environment (Turner et al. 2016), dust bathing at a carcass site would represent the riskiest possible behavior for natural exposure to inhalational anthrax. As part of a previous study by Turner et al. (2014), motion-triggered camera traps were used to monitor herbivore activity at 13 anthrax carcass sites for 20±5 mo (mean±SD). We used the behavioral data collected from this study to look for evidence of dust bathing at carcass sites. All monitored sites were locations where adult (>2 yr old) zebras died of anthrax. In selecting from among possible zebra anthrax cases to monitor, sites excluded from study were those where the carcass had been dragged around by scavengers resulting in body fluids released in multiple locations, or those that were located in disturbed areas or near a currently monitored site (Turner et al. 2014).

In addition, we used a RC 55 RapidFire (Reconyx, Holmen, Wisconsin, USA) camera trap to monitor a unique carcass site: one where an adult zebra died of anthrax on a dust bath in April 2012 (Fig. 3). This site was monitored continu-

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ously for 20 mo after death, and on occasion for 3 yr thereafter, to look for evidence of dust bathing. Although replication of such events would be desirable, this is the only carcass we detected on a dust bath.

Dust-bathing behavior of African herbivores

Dust-bathing behavior of animals was documented in central ENP from April 2012 to June 2014. Eleven motion-sensing camera traps were mounted at animal dust-bath sites to continuously monitor activity (with three sites monitored starting in 2012, eight in 2013), using PC 800 HyperFire or RC 55 RapidFire cameras (Reconyx). Sites were selected to monitor dust baths used by zebras or wildebeests and to cover an area similar to that monitored for elephants in central ENP (Fig. 4), with cameras placed at dust baths readily accessed from the road network. The closest distance between camera-monitored sites was 200 m, but these were used by different species. When the motion sensor was triggered, the cameras took a series of 10 photographs at 1-s intervals, and were programmed to continuously retrigger as long as there was activity at the site. Based on observation of tracks and feces when the cameras were placed, eight of these sites were used by plains zebra and three by blue wildebeest. These cameras monitored sites for an average of 9.5 mo (range: 4–16 mo). Behavioral data were extracted from photographs using the software Aardwolf (Krishnappa and Turner 2014). We were interested in age and sex patterns in dust bathing, but could not reliably record these from photographs. From the photo metadata, we extracted the date when dust bathing occurred. Dust bathing was defined as an animal lying down and rolling toward its back, as described for mountain zebra (*Equus zebra;* Joubert 1972) and American bison (*Bison bison;* Coppedge et al. 1999). Examples of dust baths for the three study species are shown in Figure 5. Camera-monitored dust-bath sites were all screened for *B. anthracis*.

Unlike zebra and wildebeest, preliminary observations indicated that elephants did not use specific sites for dust bathing. Thus, elephant dust bathing was documented by direct observations at four waterholes in central ENP (Okaukuejo, Ombika, Olifantsbad, and Rietfontein; Fig. 4). These observations were carried out during the dry seasons from May to October 2013 and again from May to June 2014, between 1200 and 1600 hours. Sampling was attempted at each waterhole once per week, but elephants were not always present during the observation period. During the rainy season elephants disperse into inaccessible areas and only rarely visit waterholes in the study area (Turner et al. 2013), and so the hot-wet season was not included. Elephants dust bathe by scraping the ground to collect soil, curling their trunk around it and throwing it onto their body (Rees 2002; Fig. 5). We recorded the number of elephants visiting the waterhole, the number that dust bathed, and each individual's dust-bath intensity (the number of throws of soil).

Data analysis

The daily average number of dust baths (B) for each month was calculated for each camera site from the total number (N) of dust baths observed and the number of days of data (D) recorded in each month (i) and site (j) as follows:

$$B_{i,j} = \frac{N_{i,j}}{D_{i,j}}$$

We then tested for seasonal differences in dust bathing activity using a Kruskal-Wallis test (alpha level of 0.05) for each species, comparing the daily average number of dust baths per month by season (where the months of data were grouped into the three seasons).

Elephant dust bathing was evaluated at the scale of daily observations, calculating the proportion of individuals observed during a day's sampling period that dust bathed. A linear regression was used to determine whether daily maximum temperature (as an index of seasonality) had any effect on the proportion of individuals dust bathing. We further assessed whether the intensity of elephant dust bathing correlated with maximum daily temperature using linear regression. Temperature data were downloaded from the Namibia Weather Network (2014). Data analyses were conducted using SPSS 20.0 (IBM Corp., Armonk, New York, USA).

RESULTS

Detection of *B. anthracis* from dust-bathing soils

Soil samples from 83 dust-bathing sites throughout ENP were analyzed for the presence of *B. anthracis* (Fig. 4B). Of these sites, 51 were utilized by zebras, 20 by wildebeests, seven by African elephants, two by a combination of zebras and wildebeests, and three by unknown species. Only two of the sites (2%) were positive for *B. anthracis* spores: one zebra dust-bathing site had 20 CFU/g and a site used by both zebra and wildebeest had 10 CFU/g. As a comparison, the camera-monitored site where the zebra died of anthrax (Fig. 3), which was also used

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FIGURE 3. A plains zebra (*Equus quagga*) that died of anthrax on an active dust-bath site in Etosha National Park. Photo credit: Gabriella Flacke.

as our positive control, had a concentration of 259,000 CFU/g, 1 yr after death.

Dust-bathing and anthrax-carcass sites

Dust bathing was not detected for any species at the 13 camera-monitored anthrax carcass sites (Turner et al. 2014). Similarly, the site where a zebra died of anthrax on a dust bath (Fig. 3) was never again used for dust bathing during our 5 yr of monitoring. Prior to this animal's death the site had fine powdery soil suitable for dust bathing, but after death body fluids released from the carcass were churned into the soil and compacted by scavenger activity. The soil at this site remained hard and compacted 4 yr after the zebra's death. This hardened soil, which we termed "gut cement," occurs in relatively small patches on a carcass site, but it may serve as a deterrent in choosing these locations for future dust bathing. Gut cement is a common and persistent feature of carcass sites, which we have documented at some sites lasting at least 5 yr after death (Fig. 6).

The ecology of dust-bathing behavior

The camera traps mounted at dust-bath sites were triggered a total of 31,885 times, and collected 318,850 photographs. These cameras recorded 448 zebras, 30 wildebeest, and no elephants dust bathing (Table 1). These dust-bathing triggers represented 1% of zebra, 0.07% of wildebeest, and 0% of elephant triggers. The three species seem to only rarely share dust bath sites: one time a zebra dust bathed at one of the wildebeest sites, wildebeest dust bathed five times at zebra sites, and elephants never used the sites of either species despite being observed at several of the sites. No other herbivores were observed dust bathing, despite regular visitations to the sites by springbok and gemsbok (*Oryx gazella*).

Although the number of dust baths that we recorded monthly and seasonally varied for both species, there was no statistically significant seasonal difference in the number of dust-bathing triggers by zebras (Kruskal-Wallis: χ^2=4.653, df=2, P=0.097; Fig. 7) or wildebeests (Kruskal-Wallis: χ^2=0.269, df=2,

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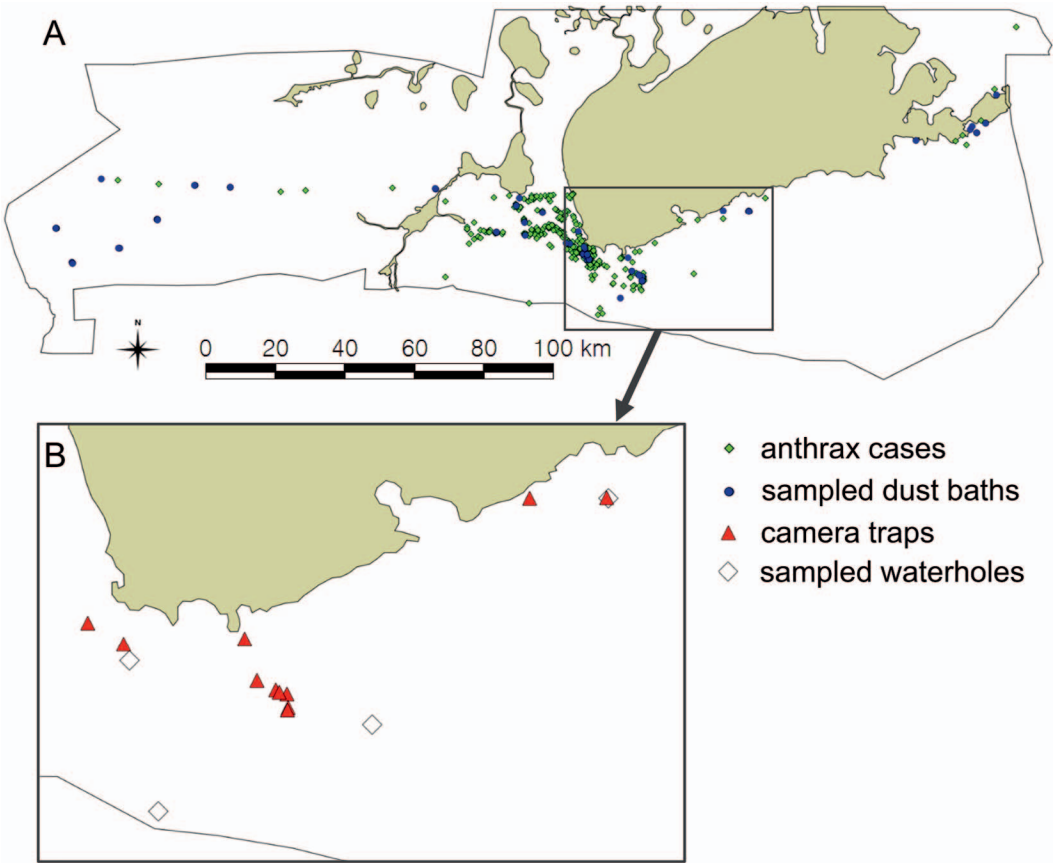


FIGURE 4. Study area, anthrax mortalities, and dust-bath sampling locations in Etosha National Park (ENP), Namibia. (A) The distribution of dust baths sampled for *Bacillus anthracis* (blue circles) and all anthrax cases recorded in ENP during and in the 5 yr leading up to the study (2007–14; green diamonds). (B) Central ENP showing locations of camera-monitored dust baths (red triangles) and waterholes sampled for African elephant (*Loxodonta africana*) dust-bathing behavioral observations (open diamonds).

P=0.870; Fig. 7). However, changes in the environment and in animal behavior with the onset of the rains made it difficult to detect dust bathing throughout the year using cameras. All cameras were placed at dust-bathing sites in the dry season when actively in use. When the rains came, all sites were overgrown with grasses. Zebra did not return to any of the monitored dust-bath sites as the next dry season approached while wildebeest reused one out of three dust-bathing sites (Fig. 2B).

For elephants, we conducted 27 d of waterhole observations where elephants were observed. On these days, a total of 285 elephants were observed of which 35 elephants (12%) took dust baths. There was no statistically significant relationship between the proportion of elephants that were dust bathing and maximum daily temperature (linear regression: R^2=0.0092, t=–0.43, P=0.671; Fig. 8A). The intensity of dust bathing also showed no statistically significant relationship with maximum daily temperature (linear regression: R^2=0.037, t=1.61, P=0.116; Fig. 8B).

DISCUSSION

In order for dust bathing to transmit inhalational anthrax infections, three conditions must be met. *Bacillus anthracis* spores

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FIGURE 5. Dust bathing by plains zebra (*Equus quagga*), blue wildebeest (*Connochaetes taurinus*), and African elephants (*Loxodonta africana*) in Etosha National Park, Namibia. A dust cloud is formed during dust baths, potentially exposing animals to soilborne pathogens. Photo credits: zebra, Holly Ganz; wildebeest, Z.R.B.; elephant, Yathin Krishnappa.

must be present at dust-bath sites, spores must be aerosolized along with soil particles during the dust bath, and aerosolized spores must be inhaled by dust-bathing animals in sufficient quantities to induce a lethal infection. We tested the first condition by quantifying *B. anthracis* spore concentrations at dust baths and found that only 2% of these sites contained *B. anthracis* spores, at very low concentrations ($\leq$20 CFU/g). The latter two conditions were not evaluated in our study, but we can assume that if present at dust baths, spores are aerosolized along with soil, and that some of them will be inhaled. Lethal doses for pulmonary anthrax are known to be orders of magnitude lower than for gastrointestinal anthrax (WHO 2008). Is it possible that these dust-bath spore concentrations,

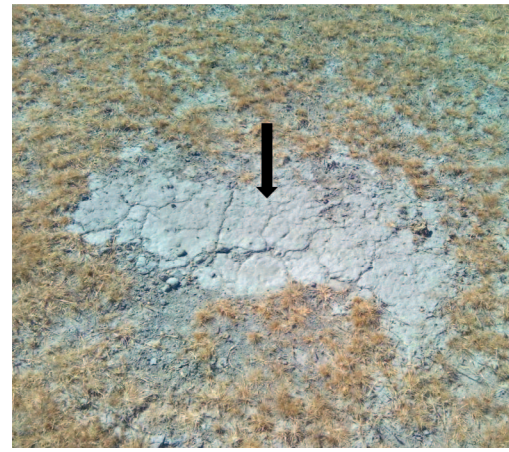


FIGURE 6. Persistent hardened soil at a zebra (*Equus quagga*) anthrax carcass site in Etosha National Park, Namibia. This "gut cement" (black arrow) image is from a 5-yr-old carcass site. Photo credit: Z.R.B.

although low, could still lead to inhalational anthrax infections?

Experimental inhalational exposure studies estimate 50% lethal doses to be around 10^4–10^5 spores across species (Bartrand et al. 2008; Taft and Hines 2012). Although we do not know the mass of soil aerosolized during dust baths, based on results of these laboratory exposure studies and our recorded spore concentrations, an animal would need to inhale on the order of 1–10 kg of dust-bath soil to receive an infectious dose. Because experimental exposure studies rely on pure spore treatments to induce infection, these may greatly underestimate lethal doses required under natural conditions. *Bacillus anthracis* spores in soil are unlikely to remain as free spores (Turnbull et al. 1998), and instead are attached to soil particles, part of the reason it can be so difficult to extract *B. anthracis* spores and DNA from soil samples (Dauphin et al. 2009). *Bacillus anthracis* spores complexed with soil particles are unlikely to remain airborne for long or to penetrate deeply into the respiratory tract (Turnbull et al. 1998). Spores do not need to reach the lungs to induce an infection; initial infection can occur in nasal-associated lymphoid tissue of the upper respiratory tract (Glomski et al. 2007). However, as the size of

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TABLE 1. Herbivore use of camera trap-monitored dust-bath locations in Etosha National Park, Namibia.[a]

Camera ID	Total triggers[b]	Days of data	Total triggers by EQ	Total triggers by CT	Total triggers by LA	EQ Db	CT Db	LA Db
12-DB2	6,331	339.8	3,628	125	0	103	3	0
12-DB4	7,522	246.5	2,385	317	0	70	1	0
12-DB5	1,272	79.2	337	34	0	4	0	0
Z13-07	3,530	69	1,494	633	6	49	0	0
Z13-08	3,299	370.9	827	10	0	199	0	0
Z13-09	2,398	298.5	847	204	0	18	1	0
Z13-11	624	145.1	150	42	0	3	0	0
Z13-16	802	186.5	124	4	1	1	0	0
W13-06	4,333	295.8	586	347	57	0	5	0
W13-10	1,586	244.9	357	54	1	1	16	0
W13-15	188	82.8	10	35	1	0	4	0
Total	31,885	2,359	10,745	1,805	66	448	30	0

[a] EQ = plains zebra (*Equus quagga*); CT = blue wildebeest (*Connochaetes taurinus*); LA = African elephant (*Loxodonta africana*); Db = number of dust baths.

[b] Triggers are the number of times a motion-sensing camera detected motion at a monitored site.

aerosolized particles increases, the dose required for a lethal exposure increases dramatically (Druette et al. 1953). Even when considering pure spore-based experimental doses as a lower bound for lethal infections, these still represent higher doses than animals could plausibly inhale while dust bathing.

If dust bathing occurred at anthrax carcass sites, animals would have been at a considerably higher risk of *B. anthracis* exposure than when dust bathing elsewhere. *Bacillus anthracis* is found in the highest concentrations in soils at anthrax carcass sites, and when spores are documented away from these primary sources of exposure, concentrations tend to be low (Lindeque and Turnbull 1994; Ganz et al. 2012; Turner et al. 2016). We found no evidence of dust bathing occurring at any of our monitored carcass sites, including one that was previously a dust bath (Fig. 3). Persistent effects of the carcass on the environment may make these sites unsuitable

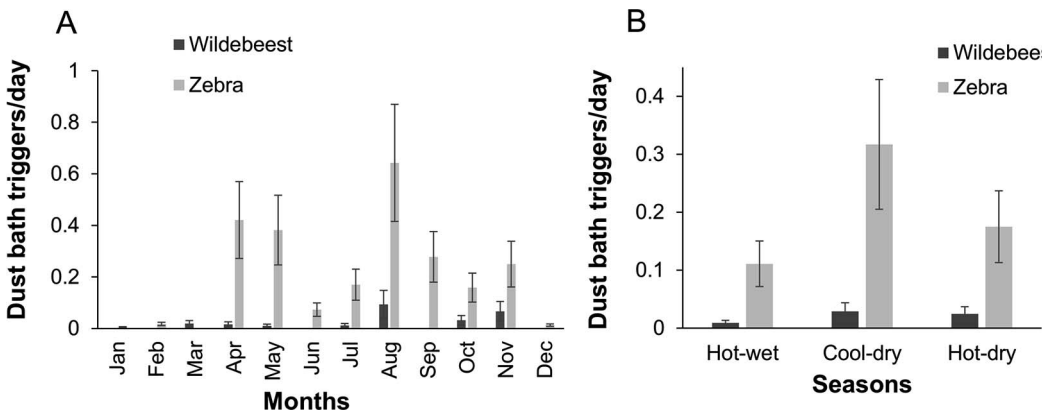


FIGURE 7. Seasonality of dust bathing by plains zebra (*Equus quagga*) and blue wildebeest (*Connochaetes taurinus*) in Etosha National Park, Namibia. Dust baths are shown (A) monthly and (B) seasonally. The number of dust baths occurring per day was recorded based on the number of triggers of a motion-sensing camera that contained dust bathing. The three seasons are hot-wet (January–April), cool-dry (May–August), and hot-dry (September–December). Error bars indicate standard error of the mean.

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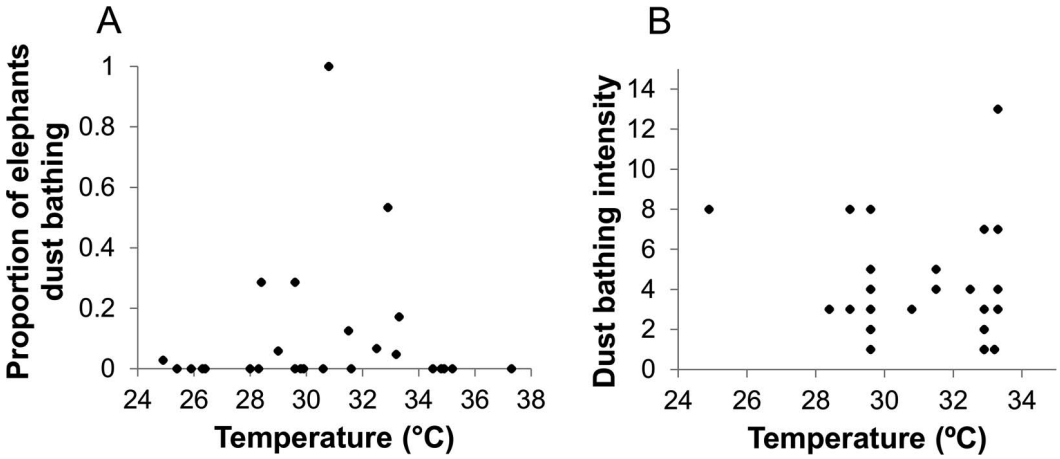


FIGURE 8. Temperature and elephant (*Loxodonta africana*) dust bathing in Etosha National Park, Namibia. (A) The relationship between maximum daily temperature and the proportion of African elephant dust bathing; n=27 sampling days. (B) Intensity of dust bathing by African elephants. Intensity was defined as the number of times an individual collected soil with its trunk and threw it on its body; n=35 elephants.

for dust bathing (Fig. 6), preventing dust-bath–related anthrax exposures at the most infectious sites.

In addition to spore dynamics in dust-bath soils, evaluating the risk of inhalational anthrax due to dust bathing benefits from an assessment of the seasonality of this behavior, given the seasonal nature of anthrax outbreaks. If dust bathing occurred primarily in a different season from anthrax cases, or if dust bathing occurred regularly throughout the year while anthrax cases were seasonal, this would have suggested that anthrax outbreaks cannot be easily attributed to dust bathing. Although we detected no obvious correlations between the seasonality of anthrax and dust bathing in any of our study species (Figs. 1A, 7B), any conclusions made must be regarded as preliminary, since we were not able to thoroughly sample dust bathing in all seasons.

Using camera traps to monitor dust-bath sites seasonally proved challenging due to the unanticipated ephemeral nature of these sites. During dry seasons, these sites contained fine, powdery soil and could be greater than 5 m in diameter. With the onset of the rains, however, the soil became clumped and the sites were covered in vegetation. If revisited by dust-bathing animals, these sites open up to be detectable dust baths into the next dry season (Fig. 2B, C), as only occurred at one of 11 of our sites. Animals are observed to dust bathe at small sites during the wet season, despite the camera-monitored sites showing very little dust-bathing activity from December to March (Fig. 7A). Although our study may have underestimated wet-season dust bathing in zebra and wildebeest, we did see evidence that dust baths can occur throughout the year, as previously noted for mountain zebra (Joubert 1972).

We found no relationship between elephant dust-bathing behaviors and temperature in the dry seasons, despite previous research on captive Asian elephants showing that dust bathing increases with temperature (Rees 2002). Instead, we recorded a greater proportion of dust bathing occurring at intermediate temperatures (29–33 C; Fig. 8A). The discordant results between the two studies could have been due to various factors. Captive elephants can be monitored continuously, but are restricted in their natural movements (Vanitha et al. 2010) and therefore may not reflect behaviors exhibited by free-ranging elephants. Elephants in groups stay and travel with the group (Wittemyer et al. 2007), thus wild individuals may be restricted in their

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freedom to dust bath if the herd is departing from the waterhole.

The seasonality of anthrax in zebra and wildebeest can be attributed to grazing at carcass sites exposing individuals to gastrointestinal anthrax primarily during wet seasons (Turner et al. 2013, 2014; Havarua et al. 2014). For elephants, most anthrax cases are observed in the hot-dry season, but elephant dust-bathing behavior in the dry seasons was not correlated with temperature. Elephants are not attracted to (zebra) anthrax carcass sites for grazing (Turner et al. 2014) and they are unlikely to be exposed to the pathogen through drinking (Turner et al. 2016). Thus, discovering the source of elephant exposure to *B. anthracis* requires further research.

Although dust bathing creates a dramatic cloud of aerosolized soil around an individual, we found that *B. anthracis* occurs in low concentrations in these soils, making inhalational anthrax transmission as a result of dust bathing highly unlikely. Despite the challenges in characterizing the full seasonality of dust bathing, we found no evidence to support an association between the seasonal timing of dust bathing and anthrax cases in any of our study species.

ACKNOWLEDGMENTS

We thank the Ministry of Environment and Tourism (MET) for granting permission to conduct research in ENP (research permit 1749/2013). We are also grateful to all the park wardens: Shayne Kötting, Immanuel Kapofi, Jeremia Lameck, and Isaskar Uahoo. We also thank all MET and Etosha Ecological Institute staff for their assistance in the field. Our gratitude goes to Tulimevava Nambahu for helping set up the cameras and Klaudia Amutenya, Claudine Cloete, Karoline Valseth, Aletta Christian, Melba Januarie, and Ashanti Namugongo for helping with data collection. Funding was provided by NSF grant OISE-1103054 (to W.C.T.).

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Submitted for publication 4 April 2017.
Accepted 26 August 2017.

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