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Reconnaissance Survey of Radioisotopes in Soil and Possible Impact on Seasonal Anthrax Outbreak at Etosha National Park, Namibia

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Abstract

Recent discovery of elevated concentrations of uranium and thorium in sediments of the Etosha Pan prompted this reconnaissance study to survey radionuclides for their possible impact on seasonal anthrax outbreaks in habitats adjacent to the pan. Plausible explanation about how animals contract anthrax is yet to be established. Because anthrax spores are non-invasive, one of the preconditions suggested for the initiation of infection is a lesion, which serves as entry point into tissue of the organism. Five samples taken from sediments at waterholes/depressions situated downwind of the pan, where the highest density of anthrax-related deaths occurs, were analysed in the laboratory using a gamma spectrometry. All sites sampled contain concentration of radionuclides higher than the crustal average, and the highest activity concentration of 93 Bq/kg ²¹⁴Bi and ²¹⁴Pb, for example, was recorded closest to the Etosha Pan. This suggests that the pan is the source of uranium, and thus possible that radionuclides are redistributed from the pan by prevailing wind, mobilized seasonally by running water and collected in pools in which animals drink. Alpha-emitting radionuclides can damage the renal, gastrointestinal and/or respiratory systems of exposed animals. Incurred damage is hypothesized as a likely mechanism under which spores are aided to enter the host for infection.

Keywords: uranium; anthrax; wildlife; Etosha National Park

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1 Introduction

Large herds of herbivores and predators in the Etosha National Park are mainly concentrated around the Etosha Pan (Fig. 1). Since the pan itself is virtually barren, the concentration of animals in its surrounding is mainly attributed to springs that dot the pan margins and sweet grass in the adjacent

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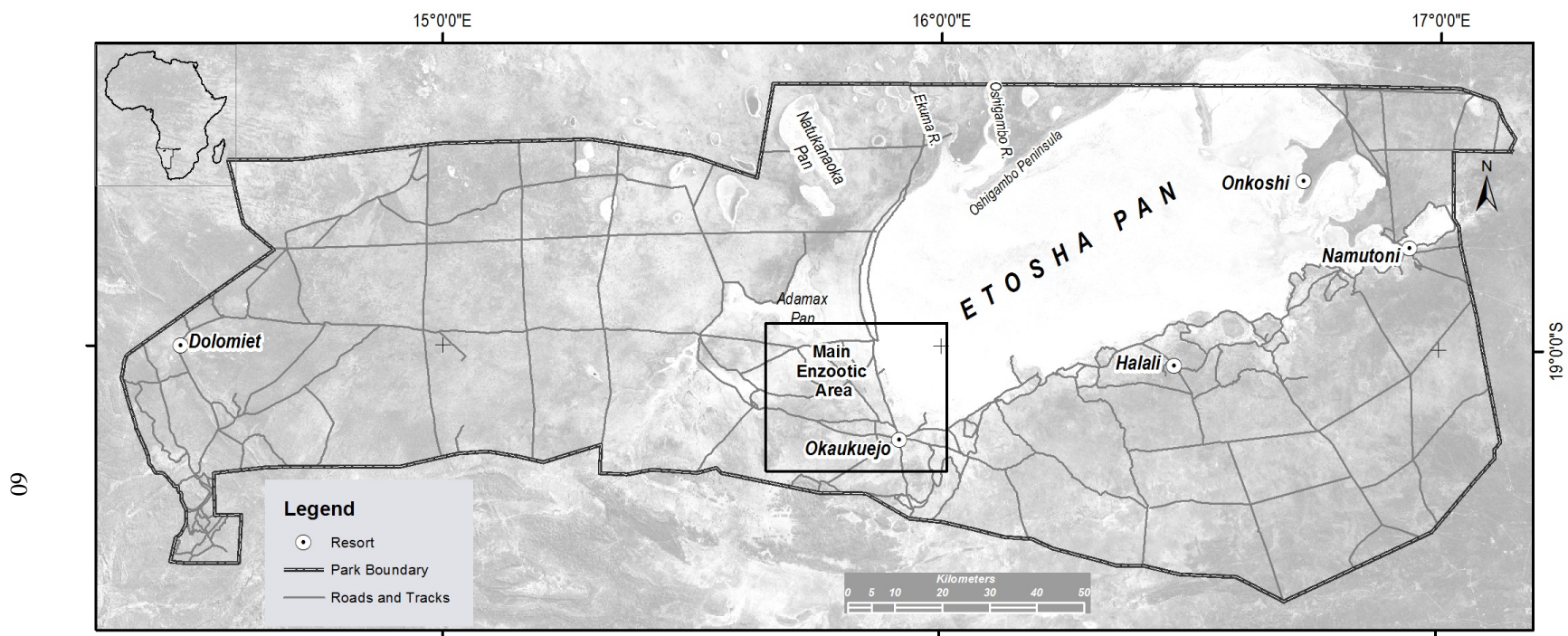


Figure 1: Landsat image, band 3, depicting the location of the study area; the area within the rectangle is depicted in more details in Fig 2.

plains (Le Roux et al. 1988). Concomitantly, a large number of ungulate herbivores, such as plains zebra, blue wildebeest and springbok, as well as elephants die enigmatically, in terms of time and space, from anthrax each year in the park (e.g. Ebedes 1976; Berry 1993). Most of these anthrax-related deaths of animals occur in the plains situated immediately on the south-western section of the Etosha Pan (Ebedes 1976; Lindeque 1991; Ganz, et al. 2009).

Recently, it became apparent that sediments around the shore of the pan have elevated concentrations of the ^{238}U isotope and its daughters. The occurrence of these radioactive isotopes in close proximity to the anthrax enzootic areas in the park motivated this reconnaissance study. Its main objective was to explore and assess the concentrations of radioactive isotopes in sediments at watering points located immediately in the south-western section of the Etosha Pan where seasonal anthrax related deaths are highest (Ebedes 1976; Lindeque 1991; Turnbull et al. 1989; Ganz et al. 2009). Results were then evaluated against the background of the unresolved entry route of the highly virulent anthrax bacterium into the organism. Alternative hypothesis for anthrax spores entrance into the host for infection is offered as a result.

1.1 Background

Anthrax is primarily a disease of herbivores (e.g. Hugh-Jones and de Voss 2002). Although dormant anthrax spores can be found at any place in the environment particularly in soil and water as a result of mechanical dispersal from sites of infected dead animals by running water, scavengers, through faeces of animals, or other agents (Jones and de Voss 2002), they may only become lethal to mammals after being reactivated and multiply after being ingested, inhaled or coming into contact with a skin lesion on a host (e.g. Beyer and Turnbull 2009; Hugh-Jones and Blackburn 2009). A high dose of spores is usually required for anthrax infection (Beyer and Turnbull 2009). However, a single spore reaching the correct site is also understood to be sufficient for an animal to contract the disease (Beyer and Turnbull 2009). In spite of concerted scientific effort spanning over a century, however, the exact mechanisms under which spores enter the blood stream to initiate infection among wildlife populations is still unknown (e.g. Ebedes 1976; Lindeque 1991; Turnbull et al. 1998; Hugh-Jones and de Voss 2002; Beyer and Turnbull 2009; Turner et al. 2013). Among the leading views, it is currently held that animals in the wild contract anthrax by ingesting the spores attached to soil, spiky grass or leaves that cause lesions in the gastrointestinal tract. A lesion is then used as the entry point into the organism (Turnbull et al. 1998). Because the bacterium is non-invasive, susceptible species with healthy integument, the intestinal mucosa and respiratory cilia are known to fend off the spores (Beyer and Turnbull 2009; Hugh-Jones and de Voss 2002) and *B. anthracis* can be detected in the faeces of animals, which remain free from the disease (e.g. Hugh-Jones and de Voss, 2002; Hugh-Jones and Blackburn, 2009).

Anthrax was first diagnosed at Etosha National Park in 1966 following enhanced research and monitoring activities (Ebedes 1976). Between 1966 and 1993, anthrax affected 10 of the 13 large herbivorous mammals (Lindeque and Turnbull 1994) and accounted for approximately 54% of total mortality in the park by 1976 (Ebedes 1976). The outbreaks take place during the rainy season and towards the end of the dry season, for plain ungulates and elephants, respectively (Ebedes 1976; Turnbull et al. 1989). Ecological incubators for anthrax spores in the park are virtually unknown. In an attempt to understand possible environmental sources of infection, Lindeque and Turnbull (1994) sampled soils and water in the enzootic areas from 23 sites not associated with known anthrax deaths and comprising springs, pans, boreholes and gravel pits. They subsequently detected *B. anthracis* in 3.3% of 92 water sources as well as 3% of 230 soil samples. More than two-

third of positive samples came from sites located in the plains of the Okaukuejo-Adamax-Okondeka triangle (Fig. 2). Overall, 70% of spores detected were obtained from soil, with a density ranging between 4 and 80 spores/g in that medium, while 1 spore/ml was found in water.

There are three naturally occurring uranium isotopes in the earth crust, namely, uranium-238 (^{238}U), uranium-234 (^{234}U) and uranium-235 (^{235}U). Amongst them, ^{234}U is the most ubiquitous element due to its mobility. This mobility is governed by the oxidative state of the element. At the surface of the earth, U^{6+} is the most common and in this oxidation state uranium is soluble. In aqueous solutions, the mobility of uranium is largely controlled by its ability to form complexes where the mobility increases with increasing pH (e.g. Ralston et al. 1986). Uranium and its daughters decay by emitting alpha, beta and gamma rays. For possible connection with anthrax, the alpha decay is the most important. Alpha particles, emitted by almost all radionuclides, have a very small traveling range (several cm in air) and high ionisation density due to their high energy (several MeV). Their ability to damage tissue is high once the radioisotope has entered the body. Most important alpha emitter in the uranium decay chain are its short-lived daughters ^{234}U (2.5×10^5 years) and ^{234}Th (24.1 days); and ^{226}Ra with its short-lived daughters ^{222}Rn (3.8 days), ^{218}Po (3.1 minutes), ^{214}Po (0.2 milliseconds) and ^{210}Po (138.4 days) (Eisenbud and Gesell 1997); the half-life of the daughters are provided in brackets. However, the most likely isotopes to enter the body are U^{6+} complex through water and ^{222}Rn through air. ^{226}Ra is moderately soluble in water and can enter groundwater by leaching from rock or sediment. ^{226}Ra then decays with alpha emission to the inert gas ^{222}Rn . ^{222}Rn is capable of seeping through water, soil, and structural barriers (Almayahi et al. 2012). The average activity concentration of uranium and ^{226}Ra in the earth's upper crust is 19 - 25 Bq/kg and 40 Bq/kg, respectively (Eisenbud and Gesell 1997; Mcraw Hill 2005).

At Etosha, elevated activity concentrations of uranium with an average of 86 Bq/kg and a maximum of 132 Bq/kg were measured (Brooks et al. 2007; Hipondoka et al. 2012). These high values were obtained from sediments fringing the pan at its north-central, north-western and western sectors. Out of 21 samples, only one sample recorded uranium concentration below 25 Bq/kg. Although no provenance study was carried out at Etosha at present, it is suspected that palaeo-rivers, such as the proto-upper Kunene and other southward flowing fluvial systems, such as the Cuvelai, brought uranium into the pan from upstream in the granitic highlands of southern Angola. Within the country, the catchment of Etosha is presently not linked to any geological deposit where mining takes place, as concluded by Oyedele et al. (2008).

2 Materials and Methods

2.1 Study area and setting

Etosha National Park is characterized by a semi-arid climate, with a wet and hot season (January to April), a dry and cold season (May to August) and a dry and hot season (September to December) (Berry 1980). Rainfall increases from 300 mm in the west to 500 mm in the east (Mendelsohn et al. 2002). During the dry season, the wind speed of the prevailing north-easterlies may exceed 20 m/s at Okaukuejo (Berry 1980). Using satellite images with high temporal resolution, Bryant et al. (2012) documented aerosol concentration blown out south-westward from the surface of the Etosha Pan. The majority of these dust plumes traversed over the study area, being located downwind of the Etosha Pan, with respect to the prevailing north-easterly wind.

Etosha Pan is a regional sump situated over the surface of the Owambo Basin. Coupled with climatic change, the pan resulted from a dried-up lake (Schwarz 1920) following the diversion of its main feeder, the Kunene River, to the coast. The timing of that diversion and subsequent formation of the pan is not well constrained and it is variously assigned to the Miocene/Pliocene (e.g. Buch et al. 1992) or Pleistocene (Stuart-Williams 1992). However, the discovery and dating of fossils of semi-aquatic antelopes (Hipondoka et al. 2006) and stromatolites (Brook et al. 2011) revealed that a series of perennial lake conditions occurred at Etosha Pan in the last 20 ka. During the Holocene, the pan also experienced phases of waning and waxing, as testified by remnants of shoreline deposits featuring as islands near the pan margins today (Hipondoka et al. 2012). Three main ephemeral rivers, Ekuma, Oshigambo, and Owambo feed into the Etosha Pan.

Le Roux et al. (1988) described the habitat surrounding the pan as short grassland and dwarf-shrubs consisting mainly of thorn bush. Animals utilize this habitat on a seasonal basis, however. During the dry season, animals are concentrated in the south and east of the pan (Tinley 1971). With the onset of rains, animals migrate to the plains located north of Okaukuejo (Tinley 1971; Ebedes 1976). These plains are the driest in the park (Hipondoka et al. 2004) and it is hypothesized that the main driver for this animals' migration is essentially to avoid moderately high rainfall in the eastern section of the park. Animal migration does not take place in drier years, as it was the case in the 2012/2013 rainy season; migration also trails delayed rains (Ebedes 1976).

Much of the Etosha Pan has highly alkaline clay soil, with pH in excess of 9 (Beugler-Bell and Buch 1997). Following the closing of artificial waterholes of Adamax, Natco and Leeubron due to overgrazing (Ebedes 1976; Berry et al. 1998) and the drying up of Wolfnes, Okondeka, a contact spring situated at the edge of the Etosha Pan, is the only perennial source of water in the study area (Figure 2). Water may accumulate in gravel pits and natural depressions during the rainy season.

2.2 Methodology

Soil samples were collected from representative locations (Figure 2) within the epizootic zone and encompasses natural (Okondeka; Eto 611 and Eto 620) and artificial (Adamax; Eto 617) waterholes, a gravel pit (Eto 615) and a natural depression (Eto 618). At Adamax, another sample was taken from a trough (Eto 616). From each site, samples were taken at a single spot from 0-15 cm depth using a spade. The sample Eto 620 was collected from a modern calcrete layer forming at Okaukuejo waterhole. Samples were then placed and sealed in one to three small, transparent plastic bags.

For gamma spectrometry analysis, around 100 g bulk samples were dried at 105 °C for two days, crushed and placed in air-tight sample holders. These were stored for four weeks prior to measurement to achieve ^{222}Rn secular equilibrium. The natural radioactivity was determined using the coaxial *n*-type Ge detector situated in the Luminescence Dating Laboratory of the University of Liverpool, United Kingdom. The 75 cm diameter detector provides a Full width at half maximum (FWHM) energy resolution of 0.87 keV at 122 keV, and an efficiency of ~32%. Samples were counted for 70-80 hours and the count rate of selected energy peaks was then compared to the certificated reference material, International Atomic Energy Agency (IAEA-375). The resulting specific activity was then transformed into concentration using the Avogadro constant (see Mauz et al. 2002 for further details).

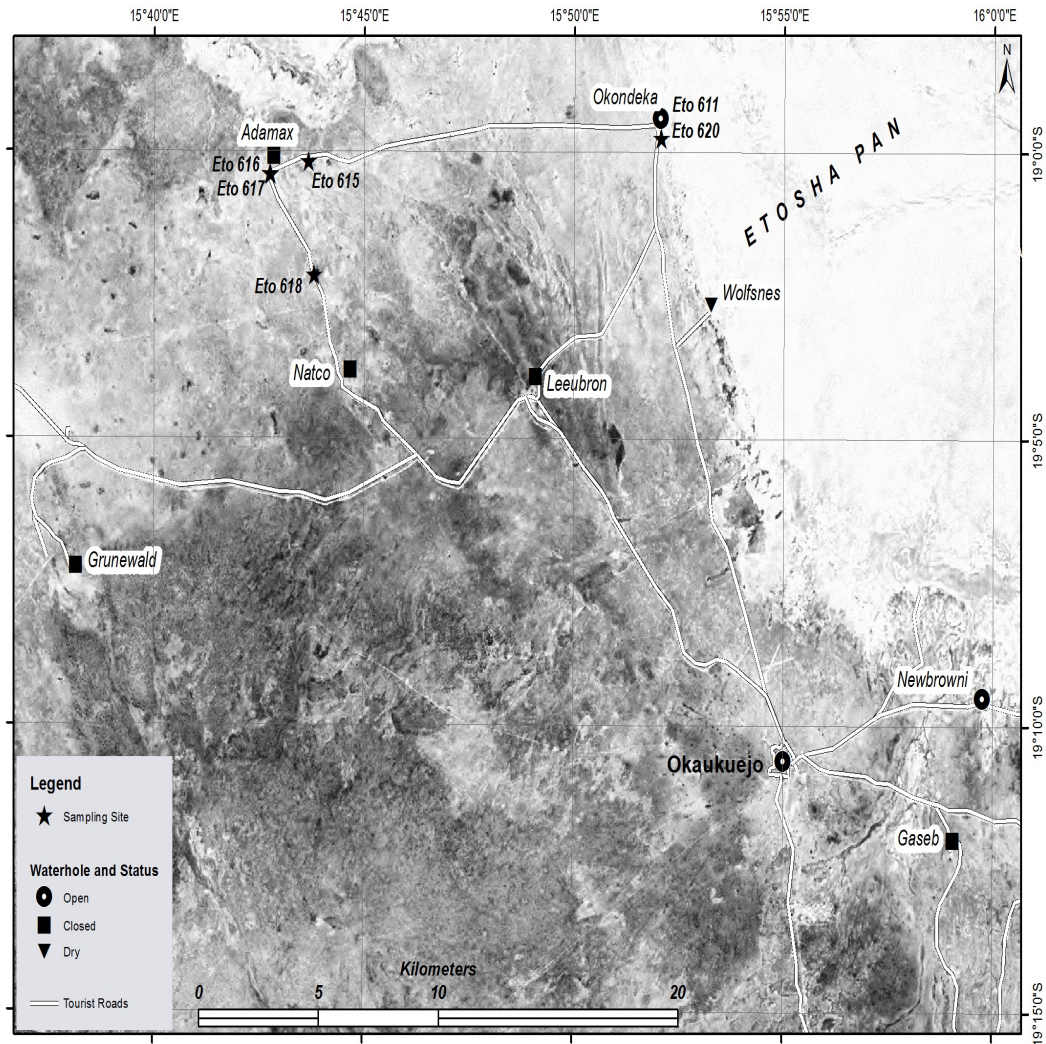


Figure 2: Location of sampling sites and types and status of waterholes in the anthrax epizootic area.

3 Results

The activity concentrations of the ^{238}U and ^{232}Th daughters, and ^{40}K in collected samples are presented in Table 1. The best estimate for the ^{238}U isotope is perhaps provided by ^{234}Th , a short-lived daughter that is in secular equilibrium with ^{238}U and ^{234}U . Some of the samples exhibit concentrations higher than the crustal average for this isotope. The activities of the daughters in the uranium chain demonstrated much higher concentrations than crustal averages, however, and it is important to note that these daughters are also key contributors to the alpha cascade of the uranium decay chain. The activity concentrations of the ^{214}Bi and ^{214}Pb daughters are significantly higher at Okondeka (Eto 611; 93 Bq/kg and Eto 620; 55 Bq/kg) as well as the natural depression (Eto 618; 63.2 Bq/kg). The average thorium (^{232}Th) is normal for all the location except for the value of the natural depression (Eto 618) which is slightly elevated. This resulted in high U/Th ratios in most of the samples, which further supports the abnormally elevated concentrations of ^{238}U and its daughters.

Table 1: Measured activity concentrations of uranium daughters, a thorium daughter and potassium in the study area.

Sample (ETO)	Location	^{234}Th	Uranium Daughters ^{226}Ra , ^{214}Pb and ^{214}Bi (weighted mean)			Thorium Daughter ^{208}Tl	Potassium ^{40}K
		Bq/kg	Bq/kg	Bq/kg	^{210}Pb Bq/kg	Bq/kg	%
611	Okondeka	56.6±1.8	63.7±3.4	93.0±2.1	116.4±18.0	25.8±0.6	0.52±0.02
615	Gravel pit	13.7±0.9	17.4±1.4	27.7±0.7	40.4±6.4	16.6±0.4	0.76±0.02
616	Adamax natural trough	14.8±1.1	21.2±1.7	32.2±0.8	80.8±12.6	21.1±0.6	1.06±0.03
617	Adamax waterhole	11.3±1.0	14.0±1.3	27.2±0.7	138.7±21.5	18.3±0.5	1.0±0.03
618	Natural depression	29.3±1.4	36.3±2.2	63.2±1.5	89.6±14.0	44.7±0.9	2.33±0.05
620	Okondeka calcrete block	18.6±0.9	30.2±1.8	55.8±1.3	59.8±9.3	1.49±0.34	0.014±0.009

With the exception of the calcrete block obtained from Okondeka waterhole (Eto 620), all samples have excessive ^{226}Ra daughters, i.e. ^{222}Rn , ^{214}Pb , ^{214}Bi . The highest activity concentrations of ^{226}Ra and its daughters were measured at Okondeka. It is however important to note that there is a substantial difference in ^{226}Ra concentrations amongst the various samples. The Adamax waterhole has a substantially higher value (138 Bq/kg) of ^{210}Pb , which is not reflected in any of the other daughters of ^{226}Ra . This might indicate a geochemical of mobility differences in the different daughters of ^{226}Ra and this is evident in the results of some of the other locations (Eto 611, Eto 615, Eto 616 and Eto 618).

4 Discussion

The activity concentrations of the ^{238}U daughters presented above revealed that sediments in the study area contain excessive alpha emitting isotopes of the uranium decay chain. Suggested reference activity concentration of uranium and its progeny vary from 22 Bq/kg to 35 Bq/kg for ^{238}U and 35 Bq/kg to 37 Bq/kg for ^{232}Th and 40 Bq/kg for radium (NCRP 1988; Eisenbud and Gessell 1997). Using these suggested reference values, the average thorium (^{232}Th) is normal for all the

locations except for the value of the natural depression (Eto 618) that is slightly elevated. This may be due to a larger catchment area from which ^{238}U is mobilized by running water into the natural depression in comparison with other sites. Radium seems to be much more abundant in all samples, which implies a high number of alpha decay of its daughters. This occurrence of elevated concentrations of uranium and its radioactive daughters in the area where anthrax mysteriously outbreaks seasonally is suggested here as a catalyst for anthrax spores to enter the blood stream of affected animal species at Etosha National Park.

Exposure to ^{238}U and its decay products are known significant risk factors for both humans and animals. Relevant to this study are the lesions affecting the renal system, (e.g. Haley et al. 1982; Gilman et al. 1998; Arzuaga et al. 2010; Vicente-Vicente et al. 2010; ATSDR 2011), gastrointestinal system (e.g. Monleau et al 2006; Paquet et al. 2006) and respiratory system (e.g. Macdonald and Laverock, 1998; Fakir et al. 2008; Bryan 2009).

The effect of uranium on animals was recently reviewed by ATSDR (2011). The kidney emerged as the most vulnerable organ in exposed animals. The other affected organ system is that of the respiratory. Chemical toxicity to the kidney usually predominates over radiation toxicity. Tissue damage can therefore be assessed in both structural and functional forms. Experimental studies in animals have further demonstrated that inhaled water soluble uranium compounds damage renal system at lower doses ($\geq 0.13 \text{ mg U/m}^3$) in comparison to exposure to insoluble uranium compounds (over 8.0 mg U/m^3). Among the animals tested for uranium inhalation studies, dogs and rabbits were the most sensitive to renal effect, followed by rats, mice, and guinea pigs, in that descending order. The adverse effect was also found to be less influenced by the duration of exposure, as summarized by ATSDR (2011).

Data on oral exposure to uranium suggest a similar trend as in the case of inhalation. Much lower doses (as low as 0.05 mg U/m^3) of soluble uranium were recorded to cause renal lesions through absorption in experimental animals (ATSDR 2011). Similarly, inhalation exposure to insoluble uranium compounds result in the deposition of some radioactive particles in the lungs, reaching systemic circulation. Insoluble compounds that remain in the lungs present a radiation hazard by irradiating alveolar tissue. Other particles ascend to the nasopharynx, with subsequent harmful effects or damage to the gastrointestinal tract through absorptive process as reviewed by Durakovic (1999). This absorption of compounds has higher toxicity effect in the gastrointestinal tract.

Radium has its own adverse health effects and it also decays into radon gas. Radon is easily picked up by animals from the air or water through ingestion (e.g. Bryan 2009). The radiological effects of radon arise from its relatively short half-life (3.8 days) and from several short-lived progeny that decay to produce high-energy alpha particles. Upon ingestion, this energy is absorbed within the respiratory system and undergoes alpha decay. The net result is damage to tissues in the respiratory system (e.g. MacDonald and Laverock 1998; Bryan 2009)

Collectively, exposure to uranium and its derivatives points to structural injury of two key internal organ systems, namely, gastrointestinal and respiratory systems. Damage to either of these organ systems has potentially serious repercussion in aiding anthrax spores to gain entry into the organism. As mentioned above, a single anthrax spore reaching a favourable spot can infect an organism, and that these spores can be ingested or inhaled by animals. Thus, it is hypothesized that the required lesion for anthrax spores to enter and infect animals at Etosha National Park is provided by ionizing radiation. Although renal damage is more common in animals exposed to uranium, animals may not necessarily be infected with anthrax through this route. This is because kidney damage by uranium and its decay products is predominantly inflicted upon its internal structure through absorption,

while the anthrax spores may not readily gain entry into the organ where scaring may have occurred. Thus, lesions in the gastrointestinal and respiratory tracts would be the most likely entry points in which anthrax spores may readily enter the organism.

From the environmental setting, it is likely that uranium is redistributed from the Etosha Pan to its leeward side by the prevailing north-easterly wind during the dry seasons. Later during the rainy seasons, surface water flowing into depressions collects uranium and its daughters, resulting in their increased concentration in water. Annual migration of animals to the leeward side of the pan coincides with this adverse environmental condition. By drinking such water, animals are then exposed to uranium and its daughters, and subsequently causing prerequisite lesions for anthrax entry into tissue as summarized above. The fact that recorded anthrax related deaths are fewer in years of low rainfall as recently experienced in 2013, partly because of the absence of animals migration to the leeward side of the pan, gives further support for this assumption. Differences in mortality peaks between plain ungulates and elephants may be related to their bodily sizes. Because of their relatively larger sizes, longer radiation exposures are required to cause fatal lesions in elephants, hence their anthrax mortality peaking later during the dry season. For plain ungulates, however, it is possible that severe damage is inflicted upon them by the middle of the rainy season, thus resulting in seasonal outbreaks in these species around that period.

5 Conclusion

Elevated concentrations of ^{238}U and its decay products in soil of the anthrax epizootic area at Etosha National Park suggests that entry of anthrax spores in the bloodstream of focal animals is aided by lesions caused by ionizing radiation in their gastrointestinal, respiratory and/or renal systems. Sediments of the Etosha Pan are considered as a local source of uranium. Aeolian and fluvial processes help mobilize ^{238}U and its daughters into depressions in the leeward side of the Etosha Pan where animals migrate during the rainy season in years with good rainfall. Subsequent water consumption from these depressions by focal animals subjects them to uranium exposure. This hypothesis of alternative pathway for anthrax infection adds a new dimension in advancing and help resolving a long-standing search for the mechanism under which animals may be infected with this virulent bacterium at Etosha National Park and possibly, allied environments. Further studies should include an investigation dealing with seasonal concentrations of radioactive isotopes in water across the park and relate results to drinking pattern and internal physiology of affected herbivores.

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