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Abstract: During a study to evaluate the impact of predation on the plains' ungulate populations in the Etosha National Park, seven cheetah *(Acinonyx jubatus)* were radio-collared. Radio-telemetry assisted in finding five of these cheetah after they had died. Four of the cheetah (57%) were confirmed to have died of anthrax, whilst the fifth, although not confirmed, possibly also died from anthrax. It is suggested that the susceptibility of cheetah to anthrax is due to their poor immunity due to lack of exposure to anthrax carcasses by being reluctant scavengers. Of seven cheetah tested, only three showed low levels of antibodies to anthrax protective antigen, the others were negative. It is speculated that cheetah are getting the disease through killing animals in the final stages of an anthrax infection.

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ANTHRAX IN WILD CHEETAHS IN THE ETOSHA NATIONAL PARK. NAMIBIA

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INTRODUCTION

Anthrax, caused by the bacterium *Bacillus anthracis*, is fundamentally a disease of herbivores. although it seems that few if any species are completely immune (Sterne 1959: Christie 1987: Turnbull 1990). Anthrax was first diagnosed in Etosha National Park (hereafter Etosha N.P.) in 1964, and has occurred on an annual basis since then. It has now been diagnosed in 14 species in Etosha N.P.

Although carnivores are known to be relatively resistant to anthrax. outbreaks of anthrax in captive carnivores are well documented, most caused by feeding on contaminated meat (Somers 1911; Lyon 1973: Ikede *et al.* 1976; Orr *et al* 1978; Jäger et al. 1990). There are also reports of carnivores succumbing to the disease in the wild (Neitz 1965; Young 1975; Mollel 1977; Turnbull et al. 1991: De Vos, Head Research, Kruger National Park, RSA, personal communication). In Etosha N.P. there have been no recorded cases of lions, hyaenas or jackals dying of anthrax. A serological survey in Etosha N.P. showed that 97% of lion and 100% of hyaena and jackal sera tested were positive for antibodies to the anthrax protective antigen toxin (PA) (Lindequc 199!; Turnbull et al. 1992). Ebedes (1976) reported two suspected cases of anthrax in cheetah, and postulated that the increased presence of anthrax, associated with gravel pits made for road building, was responsible for a cheetah population decline in the park. Until 1992, no further cases of anthrax in cheetah were reported. At that stage no cheetah sera were tested for the presence of antibodies.

Namibia has what may be the world's largest national population of free-living cheetahs. **estimated at** 2000-3000 animals, however. more than 95% of these live outside conservation areas on farmland where they frequently conflict with livestock and game farmers (Morsbach 1987). Interspecific competition between the cheetah and larger carnivores, however, appears to result in lower densities of cheetahs in protected areas with healthy populations of lion and hyaena.

Although it has been suggested that due to the marked genetic homogeneity of the cheetah (O'Brien *et al.* 1983, 1985, 1986,1987) cheetahs are exceptionally susceptible to disease (O'Brien & Evermann 1988). no significant disease-related mortality has been reported from wild cheetah populations. until the present stud!.

METHODS

Cheetah monitoring: The cheetah study in Etosha N.P. was undertaken in order to evaluate the impact of cheetah predation upon the park's plains ungulates populations. Seven cheetahs (three cheetah mothers. two young adult females, and two young adult males) were fitted with radio-collars. In order to obtain accurate estimates of the cheetahs kill frequency, or time intervals between kills, obsenations were continuous and long-term. Monitoring was of sufficient intensity and duration to ensure that all kills within the monitoring period were accounted for, either by direct observation or inference from the cats' belly size and behaviour.

From recoverable ungulate kills. after the cheetahs had finished eating. the following samples were taken: the head for aging and sexing, the femur for bone marrow analysis. and a blood smear and/or swabbing for anthrax analysis.

Confirmation of anthrax in cheetah and other species: In fresh carcases, confirmation of anthrax was done by means of a blood smear. Smears were stained with only the blue stain of CAM's Quick Stain (C.A. Milsch (Pty) Ltd, Krugersdorp, RSA), which gives results similar to M'Fadyean's polychrome methylene blue stain (M'Fadyean 1903). Blood smears were examined under oil immersion for characteristic capsulated anthrax bacilli. Terminal blood counts of *B. anthracis* in blood taken from animals shortly after death were determined using the drop count method (Lindeque 1991). When biood smears were no longer feasible because the carcase was too old, an attempt was made to isolate *B. anthracis* spores from either the carcase remains or the soil under the carcase. The principal isolation medium used was polymyxin-lysozyme-EDTA-thallous acetate agar (PLET) (Knisely 1966). according to the methodology of Carman *et al.* (1985).

Serology: For the serological survey, the method used was the Inhibition Enzyme Linked Immunosorbent Assay (ELISA) described by Turnbull et al. (1989). Protein A conjugate (Sigma P-865 1, Sigma Chemical Co., St. Louis, USA) was used in the absence of a specific antibody for cheetah. In other species this was found to work well, but gave a tine level one to two dilutions lower than when a specific antibody is used.

RESULTS

Anthrax mortality of radio-collared cheetahs in this study was very high: six out of seven sub-adult and adults died of anthrax, a mortality rate of 85.7% (Table 1). The remaining cheetah was also found dead. and although anthrax was not confirmed, it is suspect-d.

Cheetah carcases in the wild are seldom found, so the recorded mortalities in cheetahs in Etosha N.P. prior to this study have been few A computerised record of all documented mortalities from 1976-1997 reveals only 28 cheetah cases (Table 2). Prior to 1987, anthrax culturing was not performed in Etosha. N.P. and it is likely that cheetah carcases were nor tested for anthrax.

In view of the previous finding that most carnivores in the Etosha N.P. have circulating antibodies to anthrax toxin. a survey of available cheetah serum was carried out (Table 3)

DISCUSSION

The cheetah project, set up to examine the influence of cheetah on ungulate populations in Etosha N.P. during 1992-1994, provided a unique opportunity for continuous observations of radio-collared individuals. It was unfortunate that none of the victims of anthrax were under such observation during the periods prior to their deaths so that the suspect kill could be examined for anthrax, but fortunate that their carcases could be located at all. Smears from all observed cheetah kills (mostly springbok) were examined for B. *anthracis*, but no positives were found. It would seem reasonable to assume that the cheetahs dying of anthrax either scavenged off anthrax carcases (as reported by Pienaar 1196-i) in the Kruger National Park), or killed animals in the process of dying from the disease.

However. scavenging of anthrax carcases is an unlikely scenario, as the cheetah is unusual among the large carnivores for its lack of scavenging behaviour (Ewer 1973). There are only a handful of observations of cheetahs feeding from a carcase which they had not killed themselves in the wild (Pienaar 1969, Stander 1990, Caro1982 & 1994) - although they will readily eat raw meat fed to them in captivity. During this study, in nearly 9000 hours of observation, newly independent young adult cheetah siblings were observed on just one occasion to scavenge, but more experienced mothers with cubs were seen to ignore scavenging opportunities on several occasions.

The simplest explanation of why anthrax is so pernicious for cheetahs is that they have not built up immunity through exposure to anthrax carcases. This is supported by their low or undetectable levels of antibodies to anthrax protective antigen. In the Etosha environment, lions, hyaenas and black-bacheu jackals have been exposed to anthrax on a regular basis as a result of scavenging anthrax carcases, and almost all that have been tested have had substantial anti-P.&ntres(Turnbull *et al.* 1992).

That susceptibility to anthrax in carnivores appears to be a function of exposure. is indicated by lion deaths in anthrax outbreaks in the Kruger National Park. After 20 years of low incidence of anthrax. major outbreaks of the disease were experienced in areas n-here anthrax had not previously occurred. A large number of lions died from the disease, and. in fact. lion were considered among the most 'vulnerable' species during these outbreaks (De Vos, personal communication). Cheetah and wild dog were also affected. but in lower numbers, and this was athibuted to these species being less likely to scavenge (Bengis, personal communication). Kills of animals in the acute stages of anthrax (a period of only a few hours) are relatively rare, as indicated by our cheetah predation sample of over 90 anthrax-negative springbok kills. However, it probably only takes one such kill to lead to a cheetah's death, as the evidence indicates in the case of cheetah 'Hl'. Yet it is interesting that the second last cheetah to die of anthrax, 'G', had two young cubs, aged approximately ten months and thus well weaned. which did not die of anthrax.

It is also possible that homogeneity at the MHC complex of genes hampers the cheetah's ability to resist anthrax. However it is difficult to separate behavioural ecology from molecular biology though they may be related. It is postulated that the cheetah passed through a severe bottleneck of long duration or a series of bottlenecks, during the late Pleistocene large mammal extinctions of the last Ice Age, approximately 10000 years ago (O'Bnen *et al.*1985, Menotti-Raymond & O'Brien 1993). Perhaps the cheetah's non-scavenging behaviour is a post-bottleneck" strategy to avoid a disease to which it is particularly vulnerable. Conceivably a disease such as anthrax was responsible for the bottleneck, and only non-scavengers survived and reproduced. On the other hand, it is also possible that the gracile cheetah's specialized ecological niche precluded evolution of scavenging and food caching behaviour, and that its low level of genetic variation can be explained by population dynamic scenarios other than a near-extinction (Pimm *et al.*1989, Caro 1994: 353-355). The possibility also cannot be ruled out that the cheetah's lack of genetic variation is somehow adaptive or the byproduct of an adaptive process.

Population levels of cheetah in the Etosha N.P. have been a subject of concern for some 30 years and low densities have been generally attributed to competition from other predators. The high mortality rate reported in this paper confirms the suggestion by Ebedes in 1976 that anthrax may be a major population limiting factor for cheetah in the anthrax enzöotic areas of the park. Similarly, in the northern parts of the Kruger National Park where anthrax has historically been present, a survey found cheetahs to be at very low density compared to other areas with reduced incidence of anthrax (Bowland 1995). It would be of both practical and theoretical interest to evaluate the response of cheetahs to a trial anthrax vaccination programme. The results of such an experiment would indicate whether a vaccination program is a potential conservation tool for managers of anthrax-affected cheetah populations.

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REFERENCES

BOWLAND, T. (1995) Cheetahs of the Kruger National Park. *Custos* March 1995: 8-15.

CARMAN, J.A., **HAMBLETON,** P. **&** MELLING, J. (1985) *Bacillus anthracis.* In: Collins, C.H. & Grange, G.M. (Ed). Isolation and identification of micro-organisms of medical and veterinary importance. Society for Applied Bacteriology Technical Series. Academic press, London. 21: 207-214.

CARO, T.M. (1982) A record of cheetah scavenging in the Serengeti. *Afr. J. Ecol.* 20: 213-214

(1994) Cheetahs of the Serengeti plains: group living in an asocial species. University of Chicago Press. Chicago.

CHRISTIE, A.B. (1987) Infectious diseases: Epidemiology and clinical practice. 4th edition Vol 2 Churchill Livingstone 983-1003.

EBEDES, H. (1976) Anthrax epizöotics in Etosha National Park. *Madoqua.* 10:99-118.

EWER, RF. (1973) The Carnivores. Cornell University Press, Ithaca.

IKEDE, B.O., FALADE, S. **&** GOLDING, **R.R.** (1976) Anthrax in captive carnivores in Ibadan. Nigeria. *J. Wildl. Dis.* 12: 130-132.

JÄGER, H.G., BOOKER, H.H. **& HÜBSCHLE,** O.J.B. (1990) Anthrax in Cheetahs (*Acinonyx jubatus*) in Namibia. *J. Wildl. Dis.* 26:423-424.

KNISELY, R.F. (1966) Selective medium for *Bacillus anthracis. Journal of Bacteriology.* 92 784-786

LINDEQUE, P.M. (1991) Factors affecting the incidence of anthrax in the Etosha National Park. Namibia. Unpublished PhD. thesis.

LYON, D.G. (1973) An outbreak of anthrax at the Chester Zoological Gardens. *Vet. Rec.* 92: 334-337.

MENOTTI-RAYMOND & O'BRIEN, S.J. (1993) Dating the genetic bottleneck of the African cheetah *Proc. Natl. Acnd. Sci. 90:* 3172-3176.

M'FADYEAN, J. (1903) A peculiar staining reaction of the blood of animals dead of anthrax. *J. Comp* Path. 16: 35-40.

MOLLEL, C.L. (1977) A probable case of anthrax in a Serengeti lioness. *E. Afr. Wildl. J.* 15: 331.

MORSBACH, D. (1987) Cheetah in Namibia. *Cat News.* 6: 25-26. Newsletter of the IUCN SSC Cat Specialist Group. Bougy-Villars, Switzerland.

NEITZ. W.O. (1965) A checklist and hostlist of the zoonoses occurring in mammals and birds in South and South West Africa. *Onderstepoort J. Vet Res. 32: 189-374.*

O'BRIEN, S.J. & EVERMANN, J.F. (1988) Interactive influence of infectious disease and genetic diversity in natural populations. *Trends Ecol. Evol. 3:* 254-259.

O'BRIEN. S.J., WILDT, D.E., GOLDMAN, D., MERRIL, C.R. & BUSH, M. (1983) The cheetah is depauperate in genetic variation. Science. 221: 1187-l 188.

O'BRIEN, S.J., ROELKE, M.E., MARKER, L., NEWMAN, A., WINKLER, C.A., MELTZER, D., COLLY, L., EVERMANN, J.F., BUSH, M. & WILDT, D.E. (1985) Genetic basis for species vulnerability in the cheetah Science. 227:1428-1434.

O'BRIEN. S.J., WILDT, D.E. & BUSH, M. (1986) The cheetah in genetic peril. *Sci. Am.* 254(5): 68-76.

O'BRIEN, S.J., WILDT, D.E., BUSH, M., CARO, T.M., FITZGIBBON, C., AGGUNDLY, I. & LEAKEY, R.E. (1987) East African cheetahs: evidence for two population bottlenecks'? *Proc. Nat. Acad. Sci. USA. 84:* 508-511.

ORR, J.P., JOHNSTON, W.G. & MORRISON, J.R.A. (1978) Anthrax lesions in a zoo cat. *Veterinary Record. 102: 312-313.*

PIENAAR. U. de V. (1969) Predator-prey relationships amongst larger mammals of the Kruger National Park. *Koedoe. 12:* 108-176.

PIMM, S.L.. GITTLEMAN, J.L., MCCRACKEN, G.F. & GILPIN, M. (1989) Plausible alternatives to bottlenecks to explain reduced genetic diversity. *Trends Ecol. Evol. 4:* 176-178.

SOMERS, F. (1911) Anthrax amongst wild animals in captivity *The Veterinary Record.* 543-544.

STANDER. P.E. (1990) Notes on foraging habits of cheetah. *S. Afr. J. Wildl. Res.* 20(4): 130-132.

STERNE, M. (1959) Anthrax. In: Stableforth, A.W. & Galloway, I.A. (eds). Infectious Diseases of Animals. Diseases due to Bacteria. Volume1. pp16-52.

TURNBULL, P.C.B. (1990) Anthrax. In: Topley and Wilson's Principles of Bacteriology, Virology and Immunity. Vol. 2. Ed. Smith, G.R. & Eason, C.R. Sevenoaks, Kent. Edward Arnold. pp 364-377.

TURNBULL, P.C.B., BROSTER, M.G., CARMAN, J.A., MANCHEE, R.J. & MELLING, J. (1986) Development of antibodies to protective antigen and lethal factor components of anthrax toxin in humans and guinea pigs and their relevance of protective immunity *Infection and Immunity.* 52:356-363.

TURNBULL, P.C.B., BELL, R.H.V., SAIGAWA, K., MUNYENYEMBE, F.E.C.. MULENGA, C.K. & MAKALA, L.H.C. (1991) Anthrax in wildlife in the Luangwa Valley, Zambia. *Veterinary Record. 128:* 399-403.

TURNBULL, P.C.B., DOGANAY, M., LINDEQUE, P.M., AYGEN, B. & McLAUGHLIN, J. (1992) Serology and anthrax in humans, livestock and Etosha National Park wildlife. *Epidemiology and Infection.* 108: 299-313.

YOUNG, E. (1975) Some important parasitic and other diseases of lion, *Panthera leo,* in the Kruger National Park. *Jl. s. Afr. vet.* Ass. 46:181-183

Table 1 Summary of radio-collared cheetah mortalities in Etosha N.P.

Age/sex (ID)	Date collared	Date found dead	Findings
Adult female (A)	June 1992 (but previously branded in October 891	March 1993	ANTHRAX POSITIVE Carcase about 2 weeks old. Skull. mandible. pelvis and leg bones collected. Anthrax confirmed through isolation of anthrax spores from swabs taken from skull and jaw
young adult male (C)	August 1992	October 1993	POSSIBLY ANTHRAX Only bones found. No anthrax isolated. bur expert tracker found no signs of a struggle or other large predators and suggested this animal died of disease.
Sub-adult female (A1)	January 1993	November 1993	ANTHRAX POSITIVE Had been seen behaving normally in the morning apparently in good health, and was found dead in the afternoon. A blood smear was M'Fadyean positive and *B. anthracis* was found to be present in the blood at 5×10^8 cfu/ml.
Sub-adult female (H1)	March 1994 (07/03/94)	March 1994 (29/03/94)	ANTHRAX POSITIVE Found several hours after death. A blood smear confirmed anthrax. *B. anthracis* spores were isolated from the soil under the carcase at a level of 64000 spores/gram of soil. *On 27.03.94, an adult springbok carcase had been found in the vicinity. Evidence of tracks observed at the kill site and bite marks on the throat showed it was clearly killed by a cheetah. A swab taken from the bones, however, showed a high B. anthracis count (>40000 spores/ml of the swab suspension).*
Young adult male (A2)	June 1992	December 1994	ANTHRAX POSITIVE Only skull and radio-collar found. Low levels of anthrax spores isolated from swabs of skull.
Adult female (G)	February 1993	November 1995	ANTHRAX POSITIVE Carcase approximately two weeks old when found. Anthrax confirmed by PA detection assay from swabs taken from the bones
Adult female (I)	1994	June 1996	ANTHRAX POSITIVE Carcase approximately ten days old when located during aerial cracking. Anthrax confirmed by PA detection assay from swabs taken from the bones

Table 2. Cheetah mortalities recorded in the Etosha National Park since 1976

Date	Sex	Ageclass	Cause of death	Locality
77.03.10	Unknown	Immature	Unknown	Homob
77.03.10	Unknown	Immature	Unknown	Homob
77.11.07	Unknown	Cub (50cm tall)	Swatted by lion	Namutoni
78.07.03	Male	Adult	Run over by vehicle	Onguma hoek, Namutoni
78.11.08	Unknown	Sub Adult	Destroyed due to broken front leg	Namutoni
79.01.01	Female	Adult	Killed by lion	Okevi, Namutoni
79.01.01	Unknown	Cub	Cub of female killed by lion - too young to survive	Okevi, Namutoni
79.01.01	Unknown	Cub	Cub of female killed by lion - too young to survive	Okevi, Namutoni
81.01.26	Unknown	Adult	Suspected to have been killed by lions	Halali
81.02.24	Unknown	Unknown	Unknown	Twee Palms
82.12.09	Unknown	Cub	Abandoned	Namutoni
82.12.09	Unknown	Cub	Abandoned	Namutoni
82.12.09	Unknown	Cub	Abandoned cub killed by Martial Eagle	Namutoni
84.07.10	Female	Cub	Unknown	Pans edge
84.12.07	Male	Cub	Run over by vehicle	Ondongab
85.09.25	Unknown	Cub	Suspected to have been killed by lions	Sonderkop
85.10.16	Unknown	Adult	Unknown, maybe old age	Dorsland
91.01.16	Male	Sub adult	Unknown	Nomab
93.03.02	Female	Adult	Anthrax	Leeubos
93.06.17	Male	Sub adult	Capture mortality	Gemsbokvlakte
93.10.11	Male	Young adult	Suspected anthrax	Nuamses
93.11.12	Female	Sub adult	Anthrax	North of Leeubos
94.03.29	Female	Sub adult	Anthrax	South of Okaukuejo
94.12.06	Male	Young adult	Anthrax	Leeubron
95.11.27	Female	Adult	Anthrax	Gemsbokvlakte
95.12.11	Unknown	Juvenile	Starvation (cub of previous mortality)	Gemsbokvlakte
96.07.01	Female	Adult	Anthrax	Duiwelsvuur
96.12.15	Female	Adult	Killed by lion	Batia

Table 3. Cheetah sera tested for anti-PA antibodies in Etosha National Park

ID	AGE	SEX	DATE [date died]	LOCALITY	RESULT
Unknown	Unknown		88.07.13		Negative
A	Adult	_	89.10.16 92.06.25 [93.03.02]	Okaukuejo Leeubron	1:16 Negative
A1	Sub-adult *(anthrax)*	_	93.01.31 [93.11.12]	Leeubron Leeubron area	1:64 1:64
A2	Sub-adult	_	92.06.25 [94.12.06]	Leeubron	Negative
A3	Sub-adult		92.06.25	Leeubron	Negative
G	Adult	_	93.02.04 [95.11.27]	Gemsbokvlakte	Negative
C	Sub-adult	_	92.08.24 [93.10.11]	Gaseb	1:16/1:32