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Anthrax and wildlife

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Summary
Although livestock anthrax is declining in many parts of the world, with an increasing number of countries probably truly free of the disease, anthrax remains enzootic in many national parks and even in some game ranching areas. These infected areas can present a persistent risk to surrounding livestock, which may otherwise be free of the disease, as well as a public health risk. The authors use as examples the national parks in southern Africa, the Wood Buffalo National Park in northern Alberta, Canada, and the deer ranching counties in south-west Texas, United States of America, to present the range of problems, epidemiology, and control procedures. While many advances have been achieved in the understanding of this disease, research is required into the genotypic grouping of anthrax isolates, improved field diagnostic techniques, and oral vaccines, as well as to provide a better understanding of spore survival in soil and the ecology of the disease under natural conditions.

Keywords
Africa – Anthrax – Bacillus anthracis – Control – Diagnosis – North America – Wildlife.

Introduction

Anthrax is an acute to peracute, highly contagious disease of domestic and wild mammals, to which man is also susceptible. Although anthrax is primarily a disease of herbivores, all mammals are susceptible. Humans, suids and carnivores are considered incidental hosts. This disease is usually characterised by sudden death and the presence of the causal agent, *Bacillus anthracis,* in the blood and body fluids towards the terminal phase of the disease. The principal lesions are those of oedema, haemorrhage and necrosis.

The earliest record indicative of anthrax is considered to be in the Bible (Exodus, Chapters 7 to 9) which refers to the fifth and sixth plagues of Egypt, which occurred in about 1491 BC. This led to writings proposing the probable origin of anthrax to be in erstwhile Mesopotamia and northern Africa (64, 66, 67, 109). Available evidence however points to sub-Saharan Africa with its rich faunal diversity and density, as the cradle of anthrax (62, 107, 108), from whence it spread to the rest of Africa and subsequently via humans and their domestic animals, into regions such as Eurasia (67), North America (49, 111, 141) and Australia (103). During the late 19th Century and early 20th Century, anthrax was one of the foremost causes of uncontrolled mortality in livestock world-wide. In 1923, the year in which its prevalence peaked in South Africa, it was estimated that 30,000 to 60,000 animals died of anthrax (118).

The development of an effective livestock vaccine by Sterne in 1937 (114, 115), the successful application of penicillin therapy (65), and the implementation of quarantine regulations brought about a drastic decline in the occurrence of anthrax in domestic livestock in many countries throughout the world, which lessened the concern about the disease. There are, however, still a large number of countries where the situation has remained unchanged and severe epidemics still occur (56, 140). This is especially true for countries where poor socio-economic conditions occur. Anthrax spores, present in carcass remains and many old livestock burial places, are still a potential danger in most regions of the world, especially should anything happen to upset recognised practices of hygiene and control (125).

Today, even though there has been a progressive and significant global reduction in livestock cases in response to national control programmes, anthrax still occurs virtually world-wide. While surveillance is frequently defective, anthrax is now considered uncommon in most of western Europe, Canada, the United States of America (USA) and Australia, but is still

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relatively common in southern and eastern Europe, several countries of the former Union of Socialist Soviet Republics in Central Asia, southern and central America and Africa (56, 129). At the same time, it remains unchecked in wildlife in many national parks.

Because of practical difficulties encountered in vaccinating free-living wild animals, anthrax retains a place in the ecology of free-ranging wildlife in several regions of the world. In the larger free-ranging wildlife areas, with their mostly uninhibited circumstances for anthrax, the ecology of the disease can be viewed in its most natural and uncomplicated form, and continues to provide most of the baseline knowledge on the epidemiology of the disease.

Anthrax in zoological gardens and ostrich farms

Cases of anthrax in zoological gardens provide the opportunity to see each case and to investigate the causes. Unless animal care is incompetent, the deaths are obvious events with supporting documentation and witnesses. In the wild, the deaths of some species may not be so obvious or be even totally unappreciated. Similarly, in examining the circumstances surrounding such zoological garden cases it becomes equally obvious that other species, equally exposed, are unaffected. This is also a fact that is hard to appreciate in the wild natural state. Table I gives a number of examples of species reported to have been affected that one would not expect to see so in the wild – civet, coati (*Nasua nasua*), kangaroo (*Macropus* sp.), tortoise (*Emis orbicularis*), duck (*Anas* sp.), crested crane (*Balearica pavonina*), badger (*Meles meles*), ferret (*Mustela putorius f. furo*), skunk (*Mephitis mephitis*), slow loris (*Nyciticebus* sp.) and a seal – and for some, for example the birds, one may doubt the veracity. Others are merely confirmation of what has been reported in the wild, such as in lion (*Panthera leo*) and elephant (*Loxodonta africana*).

The most common causes of anthrax outbreaks in zoos follow from acquiring contaminated fresh meat from local renderers/knackers and from recycling resulting deaths. Fortunately, these events are becoming less common as the standards for zoological gardens are raised ever higher. However the smaller zoos and roadside collections are not equally well managed and therefore the situation will continue.

There are few papers on documented exposure to contaminated meat and species resistance to infection. Of course one must take such reports at face value as the exposure is not quantified; for example lions are reported as being affected but papers are rarely published where they are not affected when exposed, although it must happen. On account of their diet, carnivores, both in the wild and in zoos, may be exposed to very high doses. With this caveat in mind, Ambrosioni and Cremisini (3) reported the following species to be unaffected though exposed at the Rome zoo:

- Axolotl : *Amblystoma mexicanum*
- Carp : *Carassius auratus*, *C. carassius*, *Cyprinus carpio*, *Cy. specularis*
- Condor : *Vultur gryphus*
- Cranes : *Anthropoides paradisea*, *A. virgo*, *Grus antigone*, *G. grus*
- Dingo : *Canis lupus f. dingo*
- Eagles : *Aquila chrysaetos*, *Pithecophaga jefferyi*, *Polemaetus bellicosus*
- Fox : *Vulpes vulpes*
- Griffon : *Gyps fulvus*
- Gulls : *Larus argentatus*, *L. cachinaus*
- Hyaenas : *Crocuta crocuta*, *Hyaena striata*
- Jackal : *Canis mesomelas*
- Leopard : *Panthera pardus*
- Lion : *Panthera leo*
- Owls : *Athene noctua*, *Bubo bubo*, *Nyctea scandiaca*
- Stork : *Ciconia* sp.
- Trout : *Trota iridata*
- Vultures : *Cathartes aura*, *Gypaetus barbatus*, *Sarcoramphus papa*, *Aegypius monachus*
- Wolf : *Canis lupus*.

That scavenger species are relatively resistant to this pathogen is to be expected, if only through Darwinian pressure.

Anthrax in the African game parks

The recent works of Keim *et al*. (62) and Smith *et al*. (107, 108) allowing for the genotypic grouping of anthrax isolates suggest that the geographic origin of *B. anthracis* may be the sub-Saharan African continent. This means that prior to the arrival of man and his domestic animals, anthrax existed endemically and indigenously amongst free-living wild animals of the region. Anthrax still occurs endemically in the remnants of such pristine African wildlife areas. Major epidemics periodically flare up in African wildlife conservation areas (37, 125) such as the Queen Elizabeth National Park in Uganda, the Omo-Mago National Park in Ethiopia, the Selous Nature Reserve in Tanzania (39), the Luangwa Valley in Zambia (126), the Etosha National Park in Namibia (36, 76, 77), the Kgalagadi Transfrontier Park in South Africa and Botswana, and the Vaalbos and Kruger National Parks in South Africa (23, 27, 94).

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In the Kruger National Park, a two million ha nature reserve in South Africa, a symbiotic relationship between anthrax and the Kruger National Park complex of ecosystems exist (24). There is also no indication that anthrax is harmful to any of the wildlife in the other larger African wildlife conservation areas listed above. It is only in the smaller nature reserves and game ranches where the ecosystems have been altered by the actions of man and where the loss of a single animal could mean serious financial loss, where anthrax control is important. The areas adjacent to the Kruger National Park are mostly taken up by such smaller nature reserves and game ranches and are under threat of anthrax when an epidemic occurs in the Kruger National Park.

In multi-species African conservation areas, carcass counts and analyses demonstrate that certain herbivores are over or under-represented in relation to their population numbers and densities, indicating varying degrees of susceptibility and or behavioural vulnerability to infection. In general, the spiral horned antelope (*Tragelaphus* spp.), and buffalo (*Syncerus caffer*) are over-represented, while the Alcelaphines, zebra (*Equus* spp.) and impala (*Aepyceros melampus*) are under-represented. The important role played by blowflies (*Chrysomya* spp.) in the contamination of browse is discussed later in this chapter.

The susceptibility of different animal species to anthrax varies considerably, and most warm-blooded vertebrate species have been infected, naturally or artificially, at one time or another (117). In southern Africa, deaths due to anthrax have been recorded in at least 52 species, as indicated in Table II. Herbivores are the most susceptible, while suids, carnivores and ostriches are less susceptible, although outbreaks in these species do occur.

Anthrax in North American wildlife

Wood bison (*Bison bison*) in northern Alberta and Northwest Territories, Canada

During the summer of 1962 a large outbreak of anthrax was discovered in free-ranging bison in the Hook Lake area bordering the Slave River and north of the Wood Buffalo National Park (WBNP) (34). The size of this herd had been estimated at 1,300 head. Initially, 32 dead bison were found on 28 July in two meadows during a helicopter survey of bison habitat. There was no previous history of anthrax in the area. By 21 August, some 156 carcasses had been located in the original area. An expanded search then found a further 97 carcasses. Later a further 28 animals were also found as a result of clean-up crews travelling through areas of thick scrub. From an analysis of the carcasses, the epidemic had ceased by 19 August. As has been noted since in virtually all these outbreaks, some 80% of the dead bison were sexually mature bulls (89). The 2001 outbreak in the Park, which was discovered in late June, is an exception to the rule as there is a roughly 3:2 ratio in the carcasses between bulls and cows.

The origin of this 1962 index outbreak was never determined. However, a subsequent search of park records revealed that two park employees skinning a dead bison had suffered cutaneous anthrax in 1952. Genomic analysis of isolates from the 1962 and later outbreaks in wood bison reveal that it is a specific genotype (GT) 5 (63) that is found with few exceptions only in Canadian wood bison; the exceptions were cattle at Fort Vermilion and north of Edmonton – it is believed that these infections may have been the result of summer grazing near Fort Vermilion and the transport of latent bovine infections to these southern ranches. Anthrax in these northern areas is confined almost entirely to bison. A few dead moose (*Alces alces*) were also found towards the end of the follow-up 1963, 1964 and 1993 outbreaks. Despite widespread scavenging of carcasses by foxes, wolves (*Canis lupus*), ravens (*Corax corax*) and herring gulls (*Larus argentatus*) and the recovery of organisms from fox scat and from the digestive tract of ravens and gulls shot while feeding on carcasses, none of these species have been noted to be affected. Black bears (*Ursus americanus*) will also scavenge carcasses but it was not until 2000 that a bear was confirmed to have died of anthrax. Thus the pathogen passages with few exceptions only in bison and largely in adult wood bison, which probably explains its mutation to GT 5 (62).

The origins of the disease in this area are not known. While Dene oral tradition recounts bison die-offs back over 200 years it is more likely to have come to the WBNP between 1925 and 1928 when some 6,700 excess plains bison were moved from Wainwright in central Alberta to the southern part of the park. They also introduced brucellosis and tuberculosis. These plains bison had been acquired in earlier years from a number of small American bison herds in the adjoining northern states. An equally plausible source of the microbe was small-scale cattle operations that began arriving in the Park and Slave River Lowland areas as early as 1890. Only future calculations on known mutation rates will tell us when GT 5 was derived from the common Western North American GT 3 strain.

In the summer of 1963, a single dead bison was discovered on 27 June at Hook Lake. A series of aerial surveys were then initiated and the last of the 15 deaths was discovered on 23 July. While seemingly anticlimactic, a much larger outbreak had started in the second half of July in the Grand Detour region some 80 miles to the south of Hook Lake killing 242 bison. With the exception of two, all the carcasses were about a month old when found and buried. At the same time, another 47 affected bison were discovered in the north-east corner of the WBNP.

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Table I
Cases of anthrax in wild species in zoological gardens and ostrich farms

Name	Scientific name	Place, country	Year	Ref.
Amphibians				
Tortoise	*Emis orbicularis*	Rome, Italy	1948	3
Birds				
Crane, crested	*Balearica pavonina*	Rome, Italy	1948	3
Duck	*Anas* sp.	NOS	1923	3
Duck, mallard	*Cairina moscata*	NOS	1939	132
Ostrich	*Struthio camelus*	Grahamstown, South Africa	1908	101
		South Africa	1912	123
		Cairo, Egypt	1932	15
Carnivores				
Bear, brown	*Ursus arctos*	Tiblisi, Georgia	1926	99
Bear, polar	*Ursus maritimus*	Chester, England	1971	80
Bobcat	*Lynx rufus*	Moose Jaw, Canada	1957	87
Caracal	*Profelis caracal*	Ibadan, Nigeria	1974	58
Cat, golden	*Felis temminckii*	Glasgow, Scotland	1978	91
Cat, jungle	*Felis chaus*	India	1970-1978	98
Cat, leopard	*Prionailurus iriomotensis*	India	1970-1978	98
Cougar or puma	*Puma concolor*	Copenhagen, Denmark	1891	3
		Posen Poland	1901	72
		Rome, Italy	1948	3
		Moose Jaw, Canada	1957	87
		Glasgow, Scotland	1959	4
		Lódz, Poland	1963	110
		Skopje, Yugoslavia	1973	93
		Brno, Slovakia	NOS	68
		France	NOS	136
Dingo	*Canis lupus f. dingo*	Brno, Slovakia	NOS	68
Ermine	*Mustela erminea*	Germany	1936	2
Genet, blotched	*Genetta tigrina*	Chester, England	1971	80
Genet	*Genetta* sp.	Ibadan, Nigeria	1974	58
Jackal	*Canis* sp. (*Ther oken*)	Posen, Poland	1901	72
Jaguar	*Panthera onca*	Posen, Poland	1901	72
Leopard	*Panthera pardus*	Copenhagen, Denmark	1891	3
		Rome, Italy	1948	3
		Skopje, Yugoslavia	1973	93
Leopard, amur	*Panthera pardus orientalis*	Chester, England	1971	80
Leopard, clouded	*Neofelis nebulosa*	India	1970-1978	98
Lion	*Panthera leo*	Germany	1956	73
		Leipzig, Germany	1957	20
		Moose Jaw, Canada	1957	87
		Leipzig, Germany	1958	70
		Skopje, Yugoslavia	1973	93
		France	NOS	136
		Brno, Slovakia	NOS	68
Lynx	*Lynx* sp.	Skopje, Yugoslavia	1973	93
Lynx, European	*Lynx lynx*	Rome. Italy	1947	3
Lynx, marsh	*Lynx* sp.	Rome, Italy	1948	3
Marten	*Martes* sp.	Poland	1941	102
		Germany	1936	2
		Chester, England	1964	60

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Table I (contd)

Name	Scientific name	Place, country	Year	Ref.
Marten, stone	*Martes foina*	Copenhagen, Denmark	1891	3
Marten, American	*Martes americana*	Chester, England	1971	80
Mink	*Mustela vison*	England	1936-1937	47
		Germany	1936	2
		Ontario, Canada	1952	87
		Brno, Slovakia	NOS	68
Panther, black	*Panthera pardus*	Rome, Italy	1948	3
		Lódz, Poland	1963	110
Panther, Korean	*Panthera pardus*	Rome, Italy	1948	3
Polecat	*Mustela putorius*	Copenhagen, Denmark	1891	3
		Lódz, Poland	1963	110
		Chester, England	1971	80
Ocelot	*Leopardus pardalis*	Lódz, Poland	1963	110
Tiger	*Neofelis tigris*	NOS	1897	3
		Skopje, Yugoslavia	1973	93
		France	NOS	136
Wolverine	*Gulo gulo*	Basel, Switzerland	1955	71
Miscellaneous mammals				
Badger	*Meles meles*	Germany	1936	2
		Poland	1941	102
		Rome, Italy	1948	3
		Brno, Slovakia	NOS	68
Binturong	*Arctictis binturong*	Chester, England	1971	80
Bison, plains	*Bison bison*	Pittsburgh, United States of America	1948	82
Bison, wood	*Bison bison*	Winnipeg, Canada	1999	90
Civet	NOS	Moose Jaw, Canada	1957	87
Civet, Asian palm	*Paradoxurus hermaphroditus*	Chester, England	1971	80
Coati, ring-tailed	*Nasua nasua*	Copenhagen, Denmark	1891	3
		Chester, England	1971	80
Deer, white-tailed	*Odocoileus virginianus*	Winnipeg, Canada	1999	90
Elephant	NOS	Sofia, Bulgaria	1932	3
Elephant, African	*Loxodonta africana*	Chester, England	1964	60
Elephant, Indian	*Elephas maximus*	London, England	1927	50
Ferret	*Mustela putorius f. furo*	Poland	1941	102
		Lódz, Poland	1963	110
Fossa, Madagascar	*Cryptoprocta ferox*	Tananarive, Madagascar	1968	6
Fox	*Vulpes vulpes*	Brno, Slovakia	NOS	68
Grison	*Galictis vittata*	Chester, England	1964	60
Kangaroo	*Macropus* sp.	Alipore, India	1973	105
Loris, slender	*Loris tardigradus*	India	1975-1977	98
Mongoose,	*Galidia elegans*	Tananarive, Madagascar	1968	6
Raccoon, Madagascar ring-tailed	*Procyon lotor*	Copenhagen, Denmark	1891	3
		Posen, Poland	1901	72
		Rome, Italy	1948	3
		Moose Jaw, Canada	1957	87
		Chester, England	1964	60
		Brno, Slovakia	NOS	68
Seal	NOS	Rome, Italy	1939	16
Skunk	*Mephitis mephitis*	Germany	1936	2

NOS: not otherwise specified

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Table II
Host list and patterns of occurrence of wildlife anthrax in Southern Africa

Name	Scientific name	Pattern	Ref.
Perissodactyla			
Rhinoceros, hook-lipped	*Diceros bicornis*	R	27
Rhinoceros, square lipped	*Ceratotherium simum*	R	76
Zebra, Burchell's	*Equus burchelli*	Ep, KNP, S, ENP	23, 27, 36, 94
Zebra, Hartmann's	*Equus zebra hartmannae*	R	76
Proboscidae			
Elephant, African	*Loxodonta africana*	R, S	23, 27, 36, 94, 126
Artiodactyla			
Bushpig	*Potamochoerus porcus*	Ex	94
Warthog	*Phacochoerus aethiopicus*	Ex	27, 94
Hippopotamus	*Hippopotamus amphibius*	S	23, 27, 94, 126
Giraffe	*Giraffa camelopardalis*	R, S	27, 36, 76, 126
Antelope, roan	*Hippotragus equinus*	S	23, 27, 94
Antelope, sable	*Hippotragus niger*	R	27
Blesbok	*Damaliscus dorcas phillipsi*	Exp	23
Buffalo	*Syncerus caffer*	S, Ep	23, 27, 94, 126
Bushbuck	*Tragelaphus scriotus*	R, S	27, 94
Duiker, common	*Sylvicapra grimmia*	R	23, 27, 36, 76
Eland	*Taurotragus oryx*	R, S	23, 27, 76, 94
Gemsbok	*Oryx gazella*	S	36, 76
Grysbok, Sharpe's	*Raphicerus sharpei*	R	23, 27, 94
Impala	*Aepyceros melampus*	E, S, KNP	23, 27, 94
Klipspringer	*Oreotragus oreotragus*	R	23
Kudu	*Tragelaphus strepsiceros*	E, Ep, KNP, S, ENP	23, 27, 36, 76, 94, 126
Nyala	*Tragelaphus angasii*	R, S	23, 27, 94
Puku	*Kobus vardoni*	R	126
Reedbuck	*Redunca arundinum*	R, S	23, 94
Springbok	*Antidorcas marsupialis*	S	36, 76
Steenbok	*Raphicerus campestris*	R	23, 27, 94
Tsessebe	*Damaliscus lunatus*	R	27
Waterbuck	*Kobus ellipsiprymnus*	R, S	27, 94, 126
Wildebeest, blue	*Connochaetes taurinus*	S, KNP, Ep, ENP	27, 36, 76
Carnivora			
Badger, honey-	*Mellivora capensis*	Ex	27
Cheetah	*Acinonyx jubatus*	Ex	27, 59, 76, 94
Civet, African	*Civettictis civetta*	Ex	27, 94
Genet	*Genetta genetta*	Ex	94
Hyaena, spotted	*Crocuta crocuta*	Ex	27
Jackal, black-backed	*Canis mesomelas*	Ex	27
Leopard	*Panthera pardus*	Ex	27, 94
Lion	*Panthera leo*	R, S	27
Wild dog	*Lycaon pictus*	Ex	27, 126
Primates			
Baboon, Chacma	*Papio ursinus*	Ex	27
Vervet monkey	*Cercopithecus aethiops*	Ex	V. de Vos, unpublished findings
Struthioniformes			
Ostrich	*Struthio camelus*	Ex	36, 52, 76
Falconiformes			
Vulture, white-backed	*Gyps africanus*	Ex	27, 94

Exp : experimental
Ex : exceptional
R : rare
S : sporadic
E : endemic
Ep : epidemic
ENP : Etosha National Park, Namibia
KNP : Kruger National Park, Republic of South Africa

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During the summer of 1964, there were regular anthrax surveillance flights over the then known affected areas and following the previous schedule the first dead bison were noted in mid-July in the Grand Detour and Hook Lake areas. The peak deaths occurred between 10 and 20 July and no new carcasses were discovered after 22 July. At about this time, dead bison were seen in the Park Central region, which continued until 4 August. Carcasses were first noted in the Lake One area to the south of the Peace River on 1 August and the final carcasses were found on 10 August. After the leaves had fallen in the autumn, the areas were searched and further carcasses were found and disposed of by burning or burial. The cessation of the outbreaks was coincident with the onset of heavy and continuous rains, which lasted from mid-August through to September. There was extensive flooding with hundreds of km^2 of bison-grazing under water.

It is possible that this extensive flooding may have had an effective cleansing effect by flushing and dispersing any concentrations of deposited spores off the prairies. After three successive years of severe outbreaks, the disease had suddenly disappeared. Despite intensive surveillance flights, no single or mass deaths were noted during the summers of 1965, 1966 and 1967. Single bison carcasses were found and sampled but the tests gave negative results. Because of the advanced decomposition of some of these carcasses, the negative laboratory results were really inconclusive (Table III).

In August 1967, 120 bison died of anthrax in the Lake One region. In the following year, the disease was confirmed in only one animal. Again in 1971, a small localised outbreak involving 33 bison was found in the Hook Lake area. In 1978 there were 79 sporadic deaths in the three known endemic areas in the north but not at Lake One. Of the 79 deaths, two were cows and four were of unknown gender – decomposition and scavenging was too advanced (10). In 1991, Park Central had some 32 known bison deaths.

In late July 1993, an outbreak occurred in the Mackenzie Bison Sanctuary (MBS); 172 bison were confirmed to have died of anthrax in or around Boulogne Lake, Calais Lake, Falaise Lake, Slave Point and at Mink Lake, some 100 kms west of Falaise Lake; three moose and three bears were found dead but anthrax was only confirmed in the one sampled moose calf.

The 1,800 head in the MBS herd had been started in 1963 with 18 animals captured in the north-western WBNP. A retrospective analysis of bison serum collected before and immediately after the 1993 outbreak indicated that sero-positive animals were in the MBS before the 1993 outbreak, albeit at a very low prevalence.

The 42 sera from MBS animals bled in March 1994, showed that 39/42 had significant enzyme-linked immunosorbent assay (ELISA) antibody titres (131). This apparent high prevalence of subsequent immunity, together with the efficient searching and burning of carcasses in 1993, may explain the absence of this disease from MBS from 1994 to the present. The high prevalence of antibodies, albeit a small sample, also indicates that active widespread exposure occurred probably from tabanids which were reported in high numbers in 1993.

In July 2000, a survey team in an infrequently travelled area north of the Peace River (western border of the Lake One region) discovered eight dead adult bison on the winter road. Alerted, the park authorities initiated an aerial search and over the following weeks 100 bison and a moose were found dead with anthrax. Two bears were found dead and *B. anthracis* was isolated from a nasal swab and faeces from the first bear and from anal, nasal and mouth swabs of the second bear. A blood

Table III
Bison carcasses found during anthrax outbreaks in northern Canada

Year	Hook Lake	Grand Detour	Park Central	Lake One	Davidson Tower	MBS	Totals
1962	281	NS	NS	NS	NS	NS	281
1963	15	242	47	NS	NS	NS	304
1965	44	259	49	11	0	NS	363
1967	0	0	0	120	0	NS	120
1968	0	0	0	1	0	NS	1
1971	33	0	0	0	0	NS	33
1978	12	27	40	0	0	NS	79
1991	0	0	32	0	0	NS	32
1993	0	0	0	0	0	172	172
2000	0	NS	0	48	52	0	100
2001	12	0	0	92	0	0	104

MBS : Mackenzie Bison Sanctuary
NS : no surveys

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sample was not available from the first bear and the carotid blood sample from the second proved to be negative. Although the bears had been feeding on dead bison but without proof of a bacteraemia, one can only conclude the bears were vectors for the spores and their cause of death remains unknown. As before, the majority of affected bison were adult bulls. The affected animals were in three clusters, north and south of the Peace River and in the Sweetgrass area. Surveillance of the MBS and Hook Lake bison herds failed to reveal any other cases. The disease had not been seen in the index area before (35). In early July 2001, dead bison were found in the Lake Claire Delta, an eastern part of the area affected in 2000, and confirmed with anthrax. At the time of writing (late July 2001) 56 dead bison have been located. Aerial surveys of the Slave River Lowlands and MBS have revealed no cases north of the park. This recurrence is in contrast to the experience in the MBS in 1993 where no recurrences have been found following an active policy of burning as many carcasses as possible.

In retrospect, it is clear that anthrax in wildlife can and does occur out of sight at a low sporadic level. In North America, it leaps into blatant view usually following abundant rains in the spring, followed by a hot and dry summer. Whatever other function the rains may have, they certainly encourage an abundant hatch of tabanid flies. These will multiply the impact of any normal sporadic, summer anthrax death and facilitate animal to animal spread rapidly through any herd and to adjoining herds. This has been the epidemic pattern for bison. As bison are grazers, blowflies will play only a minor part in these epidemics but can explain moose deaths. In the Kruger National Park, outbreaks usually are associated with the driest periods of the year (winter and early spring) or during climatic dry cycles. Tabanids have not been implicated as a major transmission component in African ecosystems, but blowflies have been found to have a major role in the infection cycle of browsers.

It is also likely that the erratic nature of meteorological patterns means that when anthrax outbreaks occur, any residual effective herd-immunity from a prior exposure or exposures will have died away and left the herd at risk, though some animals may still be protected. The disease may spread explosively.

Anthrax in white-tailed deer (*Odocoileus virginianus*) in south-west Texas

This region of Texas, adjoining the Rio Grande and the Mexican border, has had cases of anthrax for a very long time. As there are no pre-Columbian records of anthrax, the pathogen probably reached Vera Cruz, Mexico, from Andalucia, Spain, via Hispaniola in the 16th and 17th Centuries. In the mid and late 18th Century, the longhorn stock were being moved northwards along the Gulf coast and eventually reached western Louisiana. They entered what is now Texas along the coastal plain and 250 miles northwest at Del Rio. These two areas, centered on the Val Verde and Uvalde and Webb counties, continue to have endemic anthrax to this day, affecting the livestock and the intensively ranched white-tailed deer.

Del Rio, Val Verde county, became the centre for the Texas mohair industry and carried about one goat per acre. As the industry collapsed through low prices in the 1960s, the ranchers responded by increasing the grazing density, which merely added to their problems as the pastures were soon grossly overgrazed. At best, the soil is only about a metre deep and overlays limestone. As the grazing degraded and the goats were removed, it was overgrown with scrub, which increased the water demands on an already dry area, further exacerbating the environmental stress and essentially locking in the environmental change. With the extra cover in what had been open pastures, the white-tailed deer, which until then had been constrained to scrub-filled draws, moved out and took advantage of the much expanded browse and protective scrub cover. The ranchers constructed game fencing around their properties and offered hunting opportunities to those willing to pay. Now each ranch has a number of feeding stations and water troughs for the deer, which are in effect feed-lotted as they are fed year-round, at a density of about five deer per acre.

On account of the perceived fear of loss of hunter income if it is publicly known that a ranch has anthrax, the deer ranchers are very reluctant to submit samples for diagnosis and official confirmation. This attitude has been reinforced by bad experiences in the severe 1974 anthrax epidemic when widespread, extended quarantine was enforced by the state authorities. The present quarantine system currently requires only ten days from the day of vaccination but unfortunately there is no vaccine delivery system suitable for deer. Unless requested by the owner, samples from deer are not sent to the laboratory for confirmation. However, as the older ranchers die or retire, many deer ranches are being divided up and sold. These new owners do arrange for samples to be sent to diagnostic laboratories, at least initially but how long this will continue is uncertain. The overall result is that though the anthrax problem is wildly recognised, and livestock anthrax is partially reported, the true dimensions of the endemicity are unclear for livestock and especially wildlife anthrax.

While regional livestock are vaccinated, this is not always consistent. Apart from any background immunity that individual deer may achieve, they are unprotected. The area is regularly tinder dry during the summer anthrax season and this means that carcasses, if found, cannot be burnt and burial is superficial in the lime-rich humus soil. Because of the difficulty of finding carcasses, scavenger activity by coyotes (*Canis latrans*), foxes, crows (*Corvus* spp.) and vultures is undisturbed, facilitating a rich sporulation and spore dispersal. Thus, even if few animals die of anthrax in a normal summer, there is an epidemiological impact beyond the raw numbers. This maintains the ongoing endemic state of the disease.

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In 1997 there was a severe epidemic of anthrax among the deer populations in parts of south-west Texas, which had no record of anthrax in living memory. Losses approached 80% on some ranches. The extent of some losses were only realised in retrospect later in the year and in 1998. Previous experience in the 1988 epidemic showed that recovery takes five years. Heavy rains in the late spring and early summer had characterised 1997. This was abruptly followed by hot and dry weather, which extended through the summer. It was noted that sick cattle were severely bothered by tabanids, which were especially abundant that year. In the area, the traditional name for these horse flies is 'charbon flies'. On a number of deer ranches, deaths were first noted among the does gathered on the fawning grounds where it spread rapidly and secondarily hit the bucks in the surrounding areas. In retrospect, it is clear that the infection was carried by tabanids from the normal initial sporadic-anthrax affected animals, possibly grazing livestock, centrifugally to surrounding ranches and then onwards to further ranches where the disease is seen only in epidemic years. Once among the deer, local spread would be via blowflies as the deer are browsers.

The years 1998 to 2000 were characterised by an increasing severe drought. However the rains recommenced in December 2000, and by February 2001, another anthrax epidemic was predicted. It started in early June in livestock, revealing that many cattle and horses were unvaccinated. By mid-month, deaths were reported in deer. At the time of writing (late July 2001) six counties around Del Rio – Uvalde are affected and, already, it is believed to be worse than the 1997 epidemic. The disease has been reported in over 40 ranches. As most cases are only known to the ranch owners and their neighbours, the true reality is still unclear. Records indicate that the highest losses occurred in July and August.

Epidemiological factors

The initiation of an outbreak or an epidemic of anthrax depends on interrelated factors, which include specific properties of the bacterium, environmental factors, factors affecting dissemination of the organism, animal densities and certain human activities (Fig. 1). With anthrax, the abilities of the bacterium to survive outside its host, to enter and successfully infect its host, and to multiply *in vivo*, are of particular importance. The basic fact to remember about *B. anthracis* is that it survives by killing. Genetically it is very stable and conservative. What it does, it does well.

The vegetative cells of *B. anthracis* are not particularly resistant to adverse environmental conditions, whilst spores are very resistant and are capable of surviving for very long periods until the opportunity to infect another host arises.

Within an infected host, *B. anthracis* spores germinate to produce vegetative forms which multiply, eventually killing the host. When conditions are not conducive to growth and multiplication, they tend to form spores but to do this need oxygen. Sporulation already starts within the live animal before oxygen levels drop below a level where growth does not occur (V. de Vos, unpublished data), but occurs primarily after an infected fresh carcass has been opened by scavengers or man, and the tissues have been exposed to the air (124).

The clotting ability of the blood of an animal suffering from septicaemic anthrax is impaired. This can result in the formation of pools of blood and tissue fluid in and around the carcass of such an animal after it has been opened. In these pools, sufficiently aerobic conditions are present which, when favourable temperatures also prevail, allow *B. anthracis* to further multiply and to sporulate (V. de Vos, unpublished data). *Bacillus anthracis* vegetative cells are 'fragile' and require protein at a protective concentration around them to prevent lysis. It is therefore a race against time to become sporulated before the protein (blood or serum) becomes diluted, dispersed or desiccated (76).

Fig. 1
Anthrax cycle in the Kruger National Park
An animal dies from anthrax and the carcass is opened by scavengers. Sporulation then takes place. Spores are disseminated by blowflies which contaminate browse in the vicinity; by vultures and mammalian scavengers which contaminate water supplies; by water run-off contaminating the grazing in the vicinity; and directly by eating from the carcass (scavengers and predators) or by chewing on old bones (herbivores with pica)
Courtesy: V. de Vos

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The bacilli (vegetative form) cannot compete with putrefactive organisms and generally die in the tissues of a carcass that has remained unopened for longer than three days at temperatures of 25°C to 30°C or higher (113). At temperatures of 5°C to 10°C, the rate of decomposition of a carcass is reduced, and *B. anthracis* can still be recovered for up to four weeks after death (139). The number of spores produced is therefore dependent on an early opening of an anthrax carcass, usually by predators, scavengers or humans. Many of these spores remain at the site of the dead animal, but some are dispersed mechanically by run-off water, scavengers or humans (Fig. 1).

Strain identification has shown that areas subject to regular epidemics have a narrowing of the diversity of strains available. For example, the Alberta bison suffer only from gene type 5; in the Kruger National Park the great majority of strains recovered are gene types 67 ('Kudu A') and 87 ('Kudu B') (63, 107).

The survival of anthrax spores in nature is dependent on their initial numbers and the nature and composition of their environment. It is believed that a variety of factors, such as climate, topography, other microbial life, certain chemicals and plant materials, may affect their survival (7, 18, 117, 122). Usually, the duration of the survival period is probably limited to not more than three years (M. Sterne, personal communication), whilst in other circumstances, such as with burial, spores were found to remain dormant and viable in nature for some 55 years (128) or even 200 ± 50 years (25).

The 'incubator area' or 'soil capability' concept of Van Ness (133, 134) propagates the theory that *B. anthracis* can survive in soil in a dynamic state in which it undergoes cycles of germination, growth and sporulation, depending on fluctuating conditions in the micro-environment. All existing evidence however points to the fact that *B. anthracis* is unable to multiply in competition with soil microbes, especially those that synthesise antibiotic substances, and therefore cannot behave as a saprophyte in nature (23, 85, 135, 142). The 'persistent spore' theory believes that the vegetative growth cycle is host-dependent and that the external environment and soil act only as intermediary vehicles in which dormant spores are conveyed to new hosts. A variation of the persistent spore theory is the 'concentrator area' concept in which host-independent growth is also discounted, but the role of calcium in spore preservation and subsequent germination requirements after dormancy are considered (33, 41, 57). It is proposed that, in environments rich in calcium, exogenous calcium buffers leaching of calcium from the calcium rich core lattice of spores, thereby enhancing preservation of the spores until optimal nutrients and conditions for outgrowth are present (33). Calcium rich environments are therefore favourable to the survival of *B. anthracis*. However, strains vary in their calcium dependency. While B strains are found only in places with high calcium levels in alkaline pH soils, A strains may favour a wider band of soils (107).

Warm climates favour the further growth and sporulation of *B. anthracis* in body fluids of opened infected carcasses with the potential for a gross contamination of the surrounding soil and vegetation. However in the Etosha National Park, contamination only occurred with 1:20 dead antelope and even then only 5% of contaminated sites had significant levels of contamination (130). In such climates, the occurrence of anthrax is closely integrated with a soil phase, leading to endemicity. In cold climates, the temperature is unfavourable for sporulation for much of the year. However, summer temperatures are high enough to trigger the disease and allow significant sporulation, even at high latitudes where daylight is also prolonged in summer. The cooler climate may explain why anthrax is not seen in the Andes over 3,000 m.

Although the mortality rate is high and few animals which contract the disease recover spontaneously (127), evidence does exist that a carrier state of latent infection can develop in individual animals and some animal species. Dormant spores of *B. anthracis* may circulate in the blood of 'black rats' (*Rattus norvegicus* NIH black) for 30 days (138). *Bacillus anthracis* was isolated from the 'abdominal' lymph nodes of apparently healthy cattle from an endemic anthrax area in Chad (97). In the Kruger National Park, several impala were first vaccinated with the Sterne spore vaccine and later challenged orally with anthrax spores. Four weeks after challenge, live and virulent *B. anthracis* organisms were isolated from the lymph nodes of these animals (23). In addition, it has been reported that a carrier state may develop in pigs that have recovered from the disease (38). Although it is not clear what role such a carrier state plays in the epidemiology of anthrax, it has been postulated that such carrier animals in an endemic anthrax area, being subjected to the occasional spore germinating which the macrophages normally mop up successfully, could develop a mild form of anthrax which unchecked when subjected to severe environmental stress, will convert into the peracute disease (39).

Discrepancies between susceptibility and prevalence to anthrax can be explained in terms of differences in behaviour, such as feeding habits and routes of infection. In the Kruger National Park, the browsers have a higher attack rate to anthrax than the grazers (23). In contrast to herbivores, pigs and carnivores are highly resistant to anthrax and the ingestion of large numbers of *B. anthracis*, such as are found in infected meat, is generally required to induce infection in them. The feeding behaviour of these animals, however, exposes them to much higher levels of infection than is the case with herbivores, and severe mortalities have been recorded in spite of their innate resistance (21, 23, 30, 58, 59, 78, 80, 94).

Age also affects the susceptibility of animals to anthrax; adults are generally more vulnerable than the young or subadults (11, 23, 31). Different behaviour patterns and feeding habits may predispose to this phenomenon, although there may also be a

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specific, but as yet, not understood, physiological basis. In the parks of Africa no sex differentiation in vulnerability or susceptibility to anthrax in any of the other species affected has been noted that could not be explained by behaviour (V. de Vos, unpublished findings; 23, 27, 30, 31). However, in the Canadian wood bison there are, as yet, no obvious exposure differences to explain the consistently higher numbers of mature bulls affected.

Apart from the role that scavengers and predators, such as coyotes, foxes, spotted hyaenas (*Crocuta crocuta*), jackals (*Canis* sp.), lions, wolves and vultures, play in the dissemination of anthrax organisms by the opening up and dismembering and dispersal of infected carcasses, they also ingest spores together with the tissues of the carcasses which are then widely disseminated in their faeces. Vultures, after gorging themselves on carcasses, usually visit watering places (23, 55, 94) where they contaminate the water by bathing and defaecating in it or the soil around its edge (Fig. 2). On occasion, they regurgitate ingested, spore-infected material into the water. Spores of *B. anthracis* occur in the faeces of white-backed vultures (*Gyps africana*) for up to two weeks after they have fed on contaminated carcasses in which bacteria have sporulated (23). It has, however, been found that vegetative forms do not survive transit through the digestive tract of vultures (55). Thus, vultures may disseminate the infection, yet they may curtail its spread by locating carcasses shortly after death and minimising contamination by rapidly consuming smaller and thin-skinned carcasses before most of the vegetative forms have had time to sporulate (22, 76). In contrast, mammalian scavengers, such as hyaenas, are more likely to chew on older carcass scraps, with a higher chance of ingesting large numbers of spores (76). Other bird species, especially those attracted by insect activity at carcasses, may also be incidental carriers of the organism. *Bacillus anthracis* has been isolated from the crops of house sparrows (*Passer domesticus*) though the risk of spread is considered to be trivial (106).

Fig. 2
Vultures at a water hole in the Kruger National Park
Courtesy: V. de Vos

Bacillus anthracis may be transmitted mechanically by vectors, such as house flies, blowflies and other arthropods including biting flies, mosquitoes and ticks (45, 46, 48, 53, 61, 86). With the exception of transmission to horses, biting flies are not considered to be of great significance in the spread of anthrax in southern Africa (52). However in North America and the foothills of the Himalayas, outbreaks are associated with seasonal tabanid activity, and following heavy rains wildlife and livestock epidemics are associated with above normal numbers of horse flies.

Ticks collected from terminally ill animals have been found to contain *B. anthracis* (V. de Vos, unpublished findings; 1, 13, 121), but they do not seem to play a significant role in the transmission of the disease as interhost transference of adult ticks is rare.

Non-biting flies can act as carriers *B. anthracis* spores (44, 79, 104) but they generally do not play an important role in the epidemiology of the disease for grazing animals, but are an important source of infection for browsers. In the Kruger National Park, blowflies (mainly *Chrysomya albiceps* and *C. marginalis*) contaminate browse fed upon by animals such as the greater kudu (*Tragelaphus strepsiceros*) (23). Blowflies feed on the body fluids from opened and unopened carcasses and, when replete or disturbed, fly off and land on vegetation in the immediate vicinity of the carcass, on which they deposit faecal, and sometimes vomit, droplets which teem with bacilli (Fig. 3). Droplets are usually deposited on the twigs or leaves of nearby bushes or trees, at a height of one to three metres above the ground, which coincides with the preferred feeding height of kudu, thus accounting for the high vulnerability rating of this species in the Kruger National Park (8, 23). For similar reasons, white-tailed deer are readily infected in North America. After a contaminated blood meal containing *B. anthracis* spores, blowflies may be life-long carriers, but vegetative cells disappear from their digestive tract within two weeks (V. de Vos, unpublished findings).

Fig. 3
Blowfly busy regurgitating a vomit droplet
Note the faecal droplet on the surface of the leaf
Courtesy: V. de Vos

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Most tropical and subtropical countries have a peak of infection during dry summer seasons and a low point six months after the peak (84, 133, 134). This also applies to anthrax in livestock in South Africa, judging by the monthly figures for anthrax outbreaks during the mid-1920s, when anthrax was at its peak in this country (137).

Outbreaks of anthrax in wildlife in the Etosha National Park, Kgalagadi Transfrontier Park and Kruger National Park in southern Africa are associated with poor soil drainage, overstocking or the concentration of game around dwindling water or forage (local overabundance) and animals suffering from environmental stress (mostly nutritional, and inter/intraspecies competition). In spite of having uniform predisposing factors, anthrax outbreaks tend to occur at different times of the year in these areas. In Kgalagadi Transfrontier Park, anthrax outbreaks peak in summer after the major rains have finished when the limited availability of water attracts and concentrates large numbers of game to man-made dams or gravel pits (V. de Vos, unpublished findings). On the other hand, in the Kruger National Park, outbreaks of anthrax typically occur in the dry season towards the end of winter and during early summer before the first major rains, when water is scarce and animals are concentrated around the remaining watering points. The onset of the rainy season dramatically terminates outbreaks in this ecosystem (23). In the Etosha National Park outbreaks in elephants peak at the end of the dry season and in the plains, ungulates are affected towards the end of the rainy season (36, 76).

In wildlife under free-ranging conditions, the general rule is an endemic anthrax situation interspersed with periodic epidemics (23, 28, 37, 39, 76, 77, 94, 126). In the Kruger National Park, these cyclic patterns of outbreaks occur approximately in ten-year intervals, or multiples thereof, are related to fluctuations in densities and concentrations of susceptible hosts (23, 28), and are recorded during climatic cycles with below average rainfall.

Any epidemic is dependent on the availability of susceptible hosts. In the Kruger National Park it was found that all the major anthrax outbreaks (1960, 1970, 1990, 1991) had essentially normal epidemic curve patterns. This is an indication that a certain number or density of susceptible animals is necessary for an epidemic to start and progress into epidemic proportions. An anthrax epidemic in a natural setting is therefore density-dependent and self-limiting. During these epidemics, anthrax killed less than 20% of its hosts, and being age-linked, left in their wake a high percentage of young animals. In a setting, such as the Kruger National Park, anthrax must therefore be considered an ideal natural culling mechanism. Another interesting phenomenon documented in the Kruger outbreaks is that as the disease passes through the population at risk, most mortalities occur on a wave-like front, with a high incidence of new cases occurring at the leading edge of the front. Behind the front, the number of cases progressively decreases, and after a few weeks only sporadic isolated mortalities are seen in the area through which the front has moved. It is not clear whether this disease pattern is due to avoidance behaviour or a protective immune response to exposure to sub-lethal doses of spores.

Signs, pathology and diagnosis of anthrax

Clinical appearance

The first sign of anthrax is finding dead animals. Herbivores such as cattle, sheep, goats and most wild ruminants mainly manifest peracute and acute symptoms. The course of the peracute clinical disease is usually less than 2 h and the acute form less than 72 h. The majority of animals are found dead without having shown signs of illness. Terminally these animals usually show opisthotonus with the fore legs rigidly extended (Fig. 4). Blood-stained fluid sometimes exudes from the nostrils, mouth and anus. Equids suffer from the acute form and oedematous swellings of the body and colic are sometimes found. Traditionally, animals that have died of anthrax do not demonstrate *rigor mortis*.

Fig. 4
Typical posture of a wild herbivore (in this case a kudu) that died from anthrax
Courtesy: V. de Vos

Carnivores, suids, and low-grade immunised animals usually show subacute to chronic symptoms, which usually extend for more than three days before recovery or death. The most frequent signs are oedematous swellings of the face, throat, neck and/or ventral parts of the body (Fig. 5). The infection may remain localised or it may progress to a septicaemia, which is usually fatal.

Sick animals will be found. The following account by Novakowski *et al.* of morbid wood bison is classic: 'The animals, head lowered, gaunt and drawn, feeding

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Fig. 5
Lion that subsequently died from anthrax
Note the swollen (oedematous) face
Courtesy: D.F. Keet

voraciously at times, were depressed and inordinately indifferent, whereas they should have been active and alert, as the outbreak occurred during the rut period. Most of the animals walked with difficulty, staggering at times, and exhibited a stiff-legged gait when running. Also a swelling of the preputial and umbilical regions was noted in many animals' (89).

The incubation period of anthrax under natural conditions is not known, but probably ranges from one to fourteen days (113).

Serological evidence seems to indicate that highly susceptible herbivores may on occasion suffer subclinical anthrax infection. In a serological survey on wildlife in the Etosha National Park in Namibia, where anthrax is endemic, it was found that naturally acquired anthrax antibodies are rare in herbivores but common in carnivores (127). This is thought to reflect the high and low mortality rates in herbivores and carnivores respectively. A divergence from this pattern has been noticed recently in the sera of Canadian wood bison following an epidemic of anthrax (40). Of 42 sera tested, 39 had specific antibodies to *B. anthracis*.

Pathology

The principal lesions in septicaemic anthrax in animals are those of widespread oedema, haemorrhage and necrosis (43, 69). Depending on the route of infection, host susceptibility and virulence of the bacteria, the nature and extent of the lesions vary at necropsy. Thus, in some carcasses, only lesions consistent with septicaemia are evident, while in others, localised necrotising lesions are found. Rarely, only enlarged lymph nodes are encountered at meat inspection (9, 38, 97, 112).

Septicaemia and bacteraemia are generally consistent features of anthrax, although the numbers of bacilli encountered in the blood may vary considerably, depending on the species (12).

In ruminants, in which the course of the disease is usually acute or peracute, the most consistent changes encountered during a necropsy include evidence of rapid post-mortem decomposition of the carcass with marked bloating soon after death; incomplete development of *rigor mortis*; oozing of blood or blood-stained fluid from the natural body openings such as the nose, mouth and anus; dark-red, poorly clotted blood; petechiae and ecchymoses throughout the carcass; degenerative changes in parenchymatous organs; extensive pulmonary oedema; excessive amounts of blood-tinged serous fluids in the peritoneal, pleural and pericardial cavities; oedema and haemorrhage in individual lymph nodes; and an enlarged pulpy spleen, the red pulp being blackish-red, soft and sometimes semifluid (Fig. 6). As suggested by the names 'splenic disease' and 'miltsiekte', severe splenomegaly is considered by many to be the most characteristic change at necropsy, but it is not a consistent feature and its absence does not rule out the possibility of anthrax. Certain species such as sheep (43), horses, pigs and impala (V. de Vos, unpublished findings) rarely develop splenomegaly.

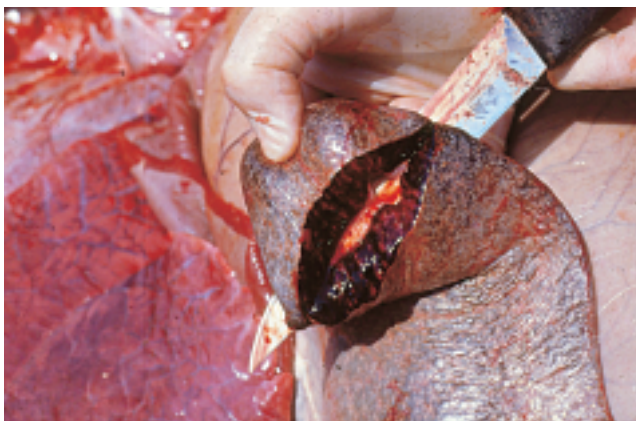

Fig. 6
Enlarged pulpy spleen of a buffalo that died of anthrax
Courtesy: V. de Vos

Most wild ruminants that have died from anthrax manifest a vasogenic brain oedema and the presence in the larger blood vessels of poorly formed and disintegrating post-mortem blood-clots, containing numerous encapsulated bacteria (69).

Equids usually suffer from septicaemic anthrax (23, 76, 94, 117).

Severe inflammatory oedemas of the soft tissues of the head, tongue and throat, stomach and intestines are characteristic features of anthrax in carnivores (18, 69, 140). In the African lion, localised lesions with no or late onset of septicaemia are

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often seen (69). These lesions are of variable severity, but are usually localised in the tissues of the face, oral cavity and the regional lymph nodes. The changes are invariably characterised by localised necrotic glossitis or stomatitis with locally extensive necrotic cellulitis of the lips and the face accompanied by severe oedema causing severe swelling of the tissues of the head (Fig. 5).

Septicaemia and bacteraemia are constant features at death, although terminal blood bacilli counts may differ according to individuals and species. A pattern was identified for species. Innate host resistance to infection by *B. anthracis* appears to be dependent on the inhibition of the initial germination and/or multiplication of the bacteria (74, 75). On this basis, it has been suggested that host species can be grouped into two categories as follows:

Species resistant to infection but once infection is established, are highly susceptible to the effects of the toxin. These species develop a low terminal bacteraemia before dying.

Species easily infected but resistant to the effects of the toxin. These species must develop a high terminal bacteraemia for sufficient toxin to be produced to cause death.

Species differences in terminal blood counts are found in wild animals from the Kruger National Park. Species, such as the greater kudu, nyala (*Tragelaphus angasi*), waterbuck (*Kobus ellipsiprymnus*) and roan antelope (*Hippotragus equinus*), consistently show a high terminal bacteraemia (Fig. 7), whilst in African buffalo and carnivores a wide variation, from virtually no organisms to numerous, occurs (12, 81). These observations correspond well with vulnerability ratings of wild animals for anthrax during outbreaks in the Kruger National Park. Animals with high terminal blood counts have been found to be highly vulnerable to anthrax (23).

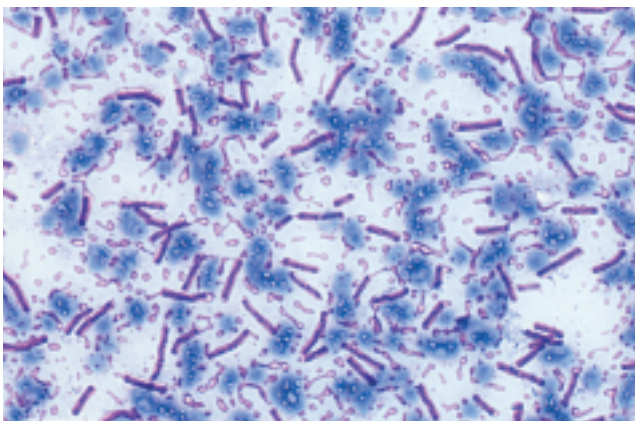

Fig. 7
Terminal blood smear of a kudu that died of anthrax
Note the capsules, which are considered pathognomonic for anthrax
Smear stained using the Giemsa staining method
Courtesy: V. de Vos

Diagnosis

The guidelines for the surveillance and control of anthrax issued by the World Health Organization (WHO) comprehensively cover diagnostic techniques (129).

The history, including clinical manifestations and necropsy findings (if, mistakenly, the carcass has been opened), is usually the first step in the diagnosis of anthrax, and should lead to further confirmatory procedures. To prevent environmental contamination, a necropsy should not be performed, if the history and clinical signs indicate anthrax. In many countries, it is forbidden by law to open a carcass suspected of being infected with anthrax. For this reason, histopathological examinations of organs or tissues as a diagnostic procedure are rarely performed. However, histologically, lesions are invariably associated with actual infection of the tissue concerned by *B. anthracis* bacteria (68).

Blood or tissue smears, best collected from oedematous swellings surrounding localised lesions or from lymph nodes regional to such lesions, such as those which occur in the region of the mesentery, throat and neck of pigs and African lions, and body extremities, such as below the hoof coronet and tips of ear or tail, should be made in an attempt to confirm the diagnosis of anthrax (14, 69, 100). *Bacillus anthracis* is readily detected in smears stained by the M'Fadyean or by Giemsa staining methods to demonstrate their capsules (Fig. 7). Their typically stained presence is considered pathognomonic for anthrax.

The absence of capsules, however, does not necessarily exclude the possibility of anthrax. As referred to above, a variation in the number of bacilli in a smear can be expected not only within animal species, but also between species; some of which consistently show high terminal bacteraemic counts, while in others few or no *B. anthracis* organisms can be detected terminally. In live animals, the organisms are seldom present in sufficient numbers in the blood to be demonstrable in blood smears, unless these are made when the disease is terminal. Low numbers can also be expected in animals which have been treated with antibiotics or in those possessing some immunity. It must further be borne in mind that, as soon as an animal dies, the anthrax bacillus in an unopened carcass undergoes changes in its morphology. The capsule commences to disintegrate and the protoplasm to degenerate, taking up the stain more and more faintly until by 24 hours after death only ghost-like bacilli are seen, the so-called 'ghost cells' or 'shadons'.

If, after the examination of a blood smear, anthrax is still suspected but unconfirmed, suitable samples (blood, mesenteric fluid, other oedematous fluid or small tissue excisions) should be collected and submitted to a laboratory for bacteriological examination; recovery is better if the blood is collected on cotton swabs and allowed to dry. This encourages sporulation and the reduction of other bacteria. Vials of blood

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are rarely positive beyond three days in transit. Soil from the immediate vicinity of the carcass (preferably underneath the carcass or where blood has spilt) has also been used with great success for culturing purposes (76, 129). The isolation and identification of *B. anthracis* from specimens originating from a relatively fresh carcass are not particularly difficult, while such attempts on material obtained from severely decomposed carcasses are often difficult or even unsuccessful (113, 139).

Isolation of *Bacillus anthracis*

Bacillus anthracis grows readily on artificial media, and when isolation is attempted from uncontaminated fresh specimens, nutrient agar can be used for this purpose, but best results are usually obtained on media containing serum or blood (preferably of horse origin). Care should be taken not to use blood or serum from animals that have been immunised against anthrax. In specimens from decomposed carcasses, from processed products of animals that have died of anthrax, or from the environment (e.g. soil), detection is complicated by a background flora of other bacteria, many of which will probably be other *Bacillus* spp., particularly, the closely related *B. cereus*. In such cases, it is necessary to use selective isolation techniques. A procedure for the selective isolation of *B. anthracis* is shown in Figure 8. The polymyxin-lysozyme-EDTA-thallous acetate (PLET) agar medium of Knisely is generally recommended (91, 126, 130). It consists of heart infusion agar as basal medium with 30 IU/ml polymixin, 300 IU/ml lysozyme, 300 µg/ml disodium ethylenediamine tetra-acetate (EDTA), and 40 µg/ml thallous acetate.

Seeded serum, blood or nutrient agar plates should be incubated for 12 h to 24 h, and PLET plates for up to 48 h at 37°C. Colonies suspected of being *B. anthracis* on the grounds of colonial morphology and tenacity, are lifted with a bacteriological needle and seeded onto a blood agar plate. An isolate with the characteristic colonial morphology and nature (matt appearance, fairly flat, white or grey-white, 2 mm to 4 mm in diameter, distinctly tacky and often having curly tailing at the edges), and which is non-haemolytic, or only weakly haemolytic, non-motile, sensitive to anthrax-specific gamma-phage and penicillin (see below), and able to produce a capsule *in vitro* in defibrinated blood or on bicarbonate media under a CO_2 atmosphere, or *in vivo* in laboratory animals is considered as *B. anthracis* (Fig. 8) (126).

For many years, the isolation and confirmation of identity of *B. anthracis* was performed in animals. This is generally unnecessary and, in line with increasing aversion to the use of animals for scientific tests, should strictly be the last resort (126).

All standard tests to assess the motility of *B. anthracis* are applicable (91). The most stringent of these is incubation of brain-heart infusion broth cultures at 22°C, 30°C and 37°C and microscopic examination to discern whether turbidity due to motility develops at any of these temperatures.

Capsule formation can be demonstrated by transferring a pin-head quantity of growth from a suspect colony to approximately 2.5 ml of sterile defibrinated blood, and incubating it at 37°C for 5 h or overnight. A smear is then made from the blood, which is stained with a capsule stain and examined microscopically. Capsule formation can also be induced by plating a suspect colony onto nutrient agar containing 0.7% sodium bicarbonate and incubating it overnight at 37°C in an atmosphere of 20% carbon dioxide (generally a candle jar will suffice).

The ability of anthrax-specific gamma-phage to induce lysis of *B. anthracis* bacilli is a phenomenon which is highly specific and is increasingly being used as a diagnostic aid in laboratories dealing with anthrax. On rare occasions, however, a phage-negative *B. anthracis* strain or a phage-positive *B. cereus* strain (126) will be found. The phage test must therefore be used in combination with other tests.

Penicillin sensitivity is tested by streaking a loopful of a colony of the organism growing on solid medium, or one obtained from a suspension of a colony prepared in peptone water, on a suitable solid medium on a plate, and then applying discs containing penicillin G (two or ten units) to its surface.

The polymerase chain reaction (PCR) has not yet become a standard method for the diagnosis of anthrax, although the technique provides the specialist laboratory with the means of rapidly confirming the presence of the virulence factor genes, and hence whether an isolate is, or is not, virulent *B. anthracis*.

Military research has developed rapid, hand-held ELISA and PCR diagnostic kits. If these could be made available commercially to park rangers and local clinicians it would facilitate the rapid and efficient disposal of infected carcasses.

For further details of laboratory isolation and confirmation of identity the reader is referred to Turnbull *et al.* (130) and Parry *et al.* (92).

Differential diagnosis

In general, all causes of sudden death and haemorrhagic septicaemia, especially in herbivores, can be confused with anthrax. Similarly, during an outbreak there is a temptation to ascribe all inactive, indifferent or limping animals to anthrax, even though, for example, tuberculosis may be known to exist in the affected herd (32).

Widespread deaths in wildlife have also been witnessed during periods of sudden severe drops in environmental temperature and during droughts. As anthrax is also associated with environmentally stressful conditions, deaths during a drought can camouflage a minor anthrax outbreak in free-living wildlife (V. de Vos, unpublished data). Sudden deaths may be associated with peracute babesiosis, chemical poisonings, toxic plants,

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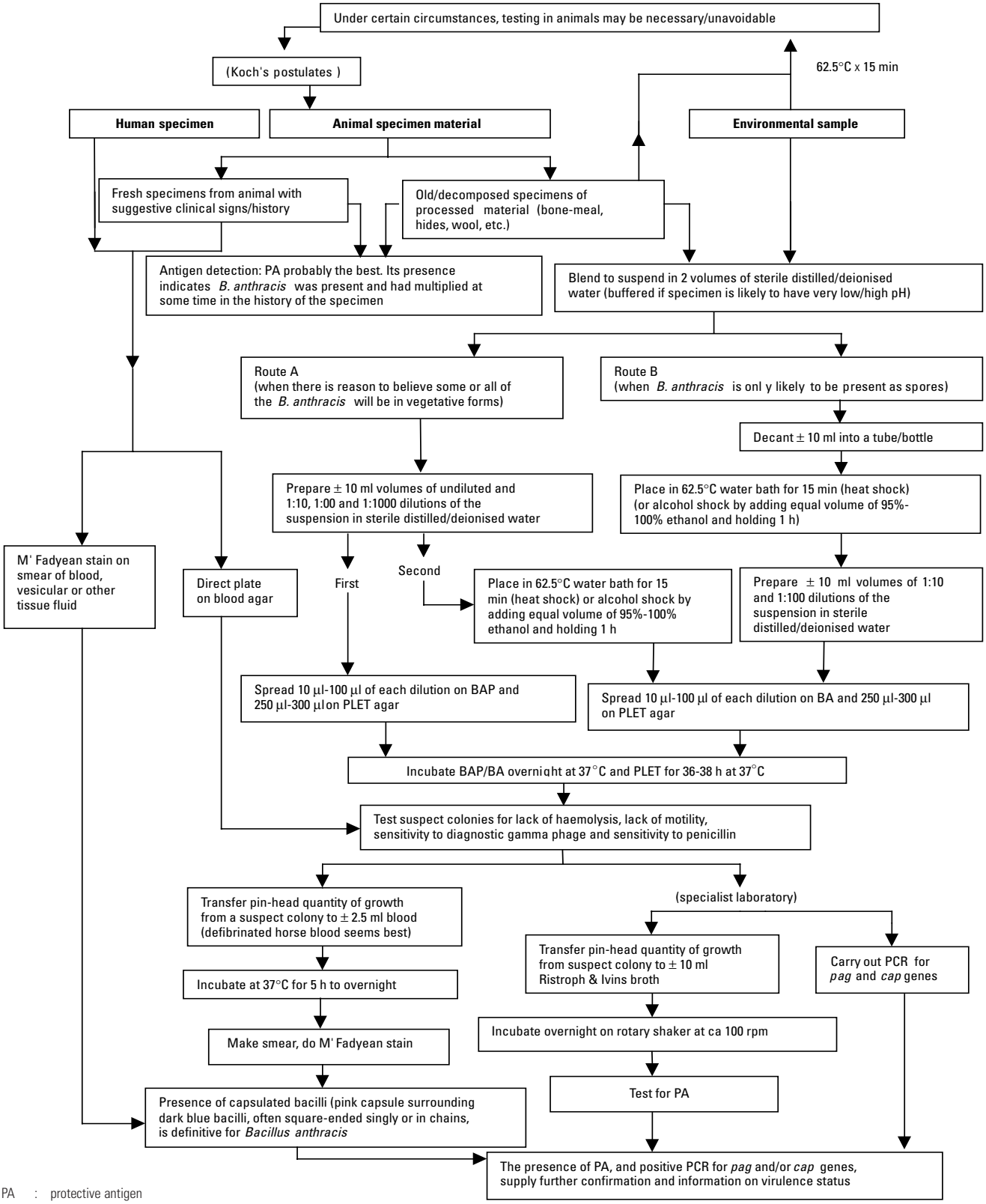

PA : protective antigen
BAP : blood agar polymyxen
PLET : polymyxin-lysozyme-EDTA-thallous acetate agar
B : blood agar
PCR : polymerase chain reaction

Fig. 8
Flow diagram of suggested procedures for isolation and identification of *Bacillus anthracis* and configuration of diagnosis
(129)

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lightening strike, Rift Valley fever and epizootic haemorrhagic disease.

Localised oedematous lesions, such as those caused by snakebite, abscessation, purpura haemorrhagica and infectious diseases, e.g. blackquarter and malignant oedema, can be confused with subacute to chronic anthrax.

Management of anthrax

Anthrax being a notifiable disease by law in most countries, control procedures are usually prescribed and enforced by Government Veterinary Services.

In the major game reserves in Africa, such as the Kruger National Park, most of the control measures, similar to those used for livestock, are difficult, if not impossible, to apply or enforce on free-ranging wildlife. In addition, anthrax being considered indigenous and a natural and integral part of the ecosystems of some of these areas (24, 107, 108), makes it debatable whether active control measures should actually be instituted. The current approach in national parks of South Africa, is to institute active control measures against anthrax only if it affects biodiversity negatively (e.g. by threatening the survival of low-density or threatened species), and/or where the actions of humans (such as fencing or the provision of artificial watering points) are providing unnatural impetus to outbreaks. Furthermore, because anthrax may also infect livestock and is a potential zoonosis, there is a moral obligation to attempt to manage the disease by reducing gross environmental contamination during major epizootics in these large conservation areas.

In addition, in the smaller parks with high contact rates with the surrounding villages and their stock, enzootic anthrax presents an ongoing livestock risk and public health problem. These small parks and ranches experience a financial loss when significant numbers of wild animals die, so disease control is necessary for their enterprise. While the large epidemics attract much attention, the disease persists from epidemic to epidemic through the annual dribble of unperceived sporadic losses and ongoing soil contamination. Consequently, while control procedures are most dramatic during an epidemic, true control may be achieved in the intervening years, which should reduce the opportunities for epidemics.

Anthrax control measures are aimed at breaking the cycle of infection and basically consist of disease surveillance, prophylactic procedures and disease regulatory actions (quarantine, immunisation, treatment, proper disposal of carcasses and disinfection). In practical terms, the objective is to reduce soil contamination, thereby preventing new outbreaks and limiting incidence when they occur. No single action, however efficient, is sufficient to control the disease.

Surveillance

These procedures must not only provide a country or province with an indication of its high-risk anthrax areas, but also function as an early warning system so that an outbreak will be identified at an early stage and combated before it assumes major epidemic proportions. Anthrax is a List B disease of the Office International des Epizooties (OIE: World organisation for animal health), and all countries that are part of this international disease surveillance network are obliged to send records of all anthrax outbreaks, including wildlife, to the OIE. Therefore, a system should be in place to rapidly confirm diagnoses; to log and map outbreaks; to maintain updated estimates of wildlife species and numbers; and to monitor environmental events associated with anthrax seasons and outbreaks. To be effective, surveillance must be proactive during anthrax seasons by looking for cases either by air or ground monitoring, with priority given to known affected areas. Airplanes or helicopters with heat-seeking devices, such as those used in combating forest fires, are very useful in locating carcasses hidden by brush and trees. Because of their 1,200 kg bulk, dead male wood bison can be found for up to seven days after death. In areas where vultures are common, field rangers should be encouraged to report incidents where sustained vulture activity is detected. Field officers can also be supplied with simple sampling kits containing two glass slides, a disposable blade, dry cotton swabs, protective gloves and a basic data sheet, for collecting peripheral blood or tissue smears from suspect carcasses.

Quarantine

Once an outbreak has been identified, it is vitally important that any possible source of infection be isolated immediately, in order to prevent further spread of the disease. The infected area (e.g. a livestock farm, nature reserve, game farm, zoo, etc.) and any surrounding farm or area which may potentially be infected should be delineated and all animal movements in and out of the area stopped. Surrounding livestock should be vaccinated and released from quarantine 10 to 14 days after vaccination. Unrestricted movements by free-ranging wildlife can be a complication and should be kept in mind when delineating a quarantine area. In the Kruger National Park, a buffalo was witnessed to have moved 20 km overnight out of an anthrax-infected area to set up a new focus of infection elsewhere (V. de Vos, unpublished findings).

Carcass disposal

The primary source of environmental contamination of anthrax spores is the carcass of an animal that has died of anthrax. To prevent sporulation, carcasses should not be opened. Carcasses should be disposed of intact and the preferred methods used in most countries that practise this method are incineration, rendering (effective controlled heat treatment) or burial. By definition, rendering is not possible with free-ranging wildlife.

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Burning is easier said than done, as suitable fuel can be scarce or the area is tinder dry. If carcasses are incinerated, the possibility should be kept in mind that viable spores of *B. anthracis* can escape with the smoke/steam above burning anthrax infected carcasses, though the risks involved are trivial compared to those of not burning. Incineration pyres should therefore be constructed in such a way that the fire rapidly takes hold and burns fiercely with as little smoke in the updraft as possible (54, 100, 130). Any contaminated soil, faeces and bedding should be added to the pyre. If the initial capital cost for equipment can be afforded (US$ 4,500), napalm is extremely efficient, fast, and very cheap. Down-directed blowtorches or portable incinerators are used for this in some countries (129). Hypothetically, collecting carcasses and burning them at a prepared central site, where surrounding scrub has been removed to reduce the risk of brush fires, would be another solution. If the park or ranch managers are unwilling to burn carcasses, the disease will eventually reappear. Finding carcasses is always a problem at the best of times – in a major push to locate dead animals during the 1990-1991 epidemic in the Kruger National Park only 49% were found based on a Lincoln Index and in the WBNP in 2000 not all dead bison were found – but when control is being attempted, proper carcass disposal is a key component of the entire control activity.

The treatment of carcasses with 5% formaldehyde, initiated almost by accident in 1993 to make use of the forest fire-fighting equipment brought by the burning crews, stops carcass scavenging and disinfects the environment in and around the body, including spilt blood. This can be done by helicopter, which means that many carcasses can be treated quickly if burning is delayed or impossible (88). The long-term environmental impacts of formaldehyde are negligible and in degradation provide nitrogen (83).

Burial is not considered a long-term solution and can result in future anthrax outbreaks (129). Spores may be brought to the surface mainly by scavengers (animals and humans), cultivation, trenching, bulldozing and soil erosion. Burial should therefore be discouraged but, if there is no feasible alternative, carcasses should be buried at a depth of two metres after being covered liberally with a mixture of one part of chloride of lime, containing at least 25% of active chlorine, to three parts of soil before the grave is filled in. In northern Canada, heavy equipment had to be brought in which was expensive.

If none of the above-mentioned methods of carcass disposal are feasible, as in remote rural areas in some developing countries, or in extensive wildlife conservation areas, the only remaining alternative is to leave the carcass unmoved and to protect it adequately from scavengers and humans (129). The rationale for this action is, as mentioned above, that most *B. anthracis* organisms within an unopened carcass do not sporulate and are inactivated by putrefactive processes. However, bloody exudate from the natural body openings may still contaminate the surrounding area, but to a much lesser degree, and should be disinfected, if possible.

Scavenger control

Spore production can be significantly reduced by stopping scavengers but this can be difficult. In parts of Africa with fuel shortages and poor infrastructures, carcasses have been covered in thorn scrub and left. For an elephant, this represents a significant amount of thorn bushes. In the Canadian MacKenzie Bison Sanctuary, it was discovered in 1993 that having the helicopter dump 400 gallons of 5% formaldehyde over the carcass very effectively discouraged all scavenging until the burning team could reach the site; presumably this will also limit the ground contamination. Obviously the volume needed for a deer would be less. Logically, the same result could be achieved by a ground crew with hand pumps using a bright colour-marked solution to designate which carcasses had been sprayed. Even if scavenging has started, spraying the carcass will limit the damage. Neither of these methods will deter hyaenas which were seen to overturn a drum with 10% formaldehyde and eat the formalin-saturated animal organs which had been in the drum.

Site disinfection

The usual procedures for livestock apply to zoo animals. Guidelines for cleaning and disinfection can be found in the WHO handbook (129).

Treatment

Antibiotics can be used as a prophylactic measure for particularly valuable animals which have been exposed to anthrax (42). This was performed successfully in the Kruger National Park when anthrax occurred in roan antelope in an enclosed camp (V. de Vos, unpublished findings). Long-acting antibiotics should be used as they are both highly effective and stress is reduced as the animals are handled only once.

Vaccination

If some way could be found to cheaply maintain effective herd immunity while the environmental contamination erodes to a safe level, one could predict that the disease itself would disappear. It is possible that this may be what was achieved at the MBS in 1993 when there was widespread infection and subsequent immunity and all but a few carcasses were effectively burnt, severely limiting ground contamination.

Most anthrax vaccines in use in the world at present utilise the toxigenic, non-capsulating ($pX01^+/pX02^-$) *B. anthracis* 34F isolated in 1937 by Sterne (114, 115). The live spore Sterne vaccine is, for all practical purposes, non-pathogenic in most animal species (26, 29). It retains a degree of virulence for

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certain species, such as goats and llamas (*Lama glama*) (17, 116). In such species, two inoculations administered one month apart, with the first being one quarter of the standard dose and the second being the full standard dose, are recommended (129). In equids which are slow to react, two standard doses one month apart and a single annual booster thereafter are recommended (5). In the other species, a single inoculation provides effective immunity for about a year (116), with annual revaccination being generally considered to be adequate to ensure permanent protection in most situations (119, 120). Effective immunity generally develops within a week of vaccination, although it may take a month or more in equids (117).

Earlier investigations were in agreement that parenteral treatment and vaccination of large populations of free-ranging wildlife are largely impractical, cause unacceptable stress and losses, are highly expensive and in some instances are almost impossible (18, 51, 96). Nevertheless, herd animals such as Burchell's zebra (*Equus burchelli burchelli*), blue wildebeest (*Connochaetes taurinus*), African buffalo and American bison which can be corralled or captured, can be vaccinated (19). Long-stemmed, hand-held automatic vaccinating syringes are used from the sides of a shute (95) but, needless to say, the strength and the wild and unruly nature of these animals makes this method both hazardous and difficult. An aerial method of immunising free-ranging wildlife was subsequently developed with projectile darts containing the vaccine (26). A helicopter brings the operator within effective firing range of the animal to be darted. This method was later improved by the use of a ballistic implantation method (29). It consists of a gun-like device capable of accurately delivering, over short distances, lightweight biodegradable implant projectiles (bio-bullets) containing the vaccine. Only a proportion of the target species can be protected in this manner. If kept in mind that an anthrax outbreak in the Kruger National Park phases out when about 20% of the host animals have been killed (see above), then the immunisation of a proportion of the population can actually stop an epidemic. This method has been used very successfully in smaller game ranches adjacent to the Kruger National Park (29).

While the call for new vaccines may be limited in African wildlife, there is an urgent need for a cheap and highly effective oral vaccine elsewhere and especially for ranched deer. This vaccine should protect the susceptible species while the environmental contamination erodes. To do this, oral vaccination – darting animals is too expensive except for very valuable species – must achieve >80% herd immunity or whatever is the agreed minimum threshold level based on species and density. On the other hand, the field pathogen can infect animals when imbibed at contaminated water holes and it will survive successfully with only a trivial mortality rate. Thus, any putative oral Sterne-type vaccine, whether delivered in feed, as for the Texas white-tailed deer, or in water for less pampered species, must survive for at least a week with a high probability of a controlled vaccinal infection. Thirdly, it must be easy to deliver in a manner that assures effective take-up by all in a herd or group, the alphas and the omegas, and risk-free to the operators.

Water controls

Because African vultures bathe in watering places thereby contaminating the water with spores, natural waterholes have been replaced by concrete drinking troughs in which the water can be disinfected or from which it can be drained (18, 23, 94).

Research needs

While many of the basic questions about *Bacillus anthracis* are common to all susceptible species, in the area of wildlife a few stand out.

Genotypic analysis

Anthrax isolates need to be analysed in order to provide further insight into the origin, evolution, persistence, and complex web of causation of the disease. Initial studies seem to point to sub-Saharan Africa as the origin of the disease with the implication that the disease may be in symbiosis with the larger wildlife ecosystems.

The development of a field diagnostic kit

By the nature of the situation, wildlife die far from diagnostic facilities and control decisions have to be made without delay. As animals may be found days or weeks after death, the numbers of viable spores may be few. The new methods utilising ELISA and PCR need to be mobilised for rapid, cheap, robust and rigorous field kits.

The development of an effective oral vaccine

An effective oral vaccine would offer benefits to livestock but for wildlife, research must extend beyond the problems of vaccine development to those of how it can be best and most efficiently and cheaply delivered to each target species. In addition, the normal seroprevalence of antibodies before and after outbreaks needs clarification.

The role of soil in the life cycle of anthrax

Little is known about how and why some soils support spore survival and others do not. The old traditions of endemic areas having soils of high pH and high calcium are still relevant but how and why are still conundra. Similarly, why even sporadic disease is unusual in acid soils should be studied.

The factors underlying age susceptibility

Why the disease is consistently seen most often in mature adults should also be examined.

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Seasonal triggers for sporadic and epidemic outbreaks in different species in the various parks

While various factors, such as rainfall, soils, temperature, animal density, vectors, are recognised, their separate and synergistic quantitative impacts are not known. If these were better understood, the disease incidence could be predicted and proactive measures taken in the appropriate areas judged to be at risk.

A study of the ecology of anthrax in the different wildlife reserves

Wildlife reserves offer unique opportunities to study the disease in its natural surroundings. Whether the disease poses a threat to these and adjoining areas, or whether it is a necessary element of these ecosystems and only assumes epidemic proportions when these areas are under stress should be clarified. This is apparently true for the Kruger National Park, but the question should be raised as to whether or not it also applies to the other nature reserves.

Conclusions

Anthrax is an old disease which probably first emerged in wildlife populations in southern Africa. With the domestication of ruminants, the disease moved out of Africa to the rest of the world, affecting livestock and wildlife. Meanwhile, it has continued to occur in African wildlife, which are now constrained increasingly to parks. In the large parks in southern Africa and elsewhere in the world, it is one of a spectrum of natural culling mechanisms. However the medium-sized and smaller parks and game ranches interact with local populations and their livestock and therefore anthrax can present risks and thus needs to be controlled. While anthrax can be controlled and even eradicated in livestock, control in wildlife, especially in the large wildlife reserves, is not yet possible. If anthrax is to be controlled, in-depth studies are required of ecological interactions with its biotic and abiotic environments. Better diagnosis and the development of suitable vaccines and delivery systems, where vaccination is necessary, are also important.

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Fièvre charbonneuse et faune sauvage

M.E. Hugh-Jones & V. de Vos

Résumé

La fièvre charbonneuse, dont l'incidence chez les animaux d'élevage décroît dans de nombreuses régions du monde, un nombre croissant de pays étant probablement indemnes, reste toutefois présente à l'état enzootique dans de nombreux parcs nationaux ainsi que dans certains élevages de gibier. Les zones d'enzootie représentent un risque persistant pour les élevages de la région, qui peuvent par ailleurs être indemnes de la maladie, ainsi que pour la santé publique. Les auteurs examinent la situation observée dans les parcs nationaux du sud de l'Afrique, dans le Wood Buffalo National Park dans le nord de l'Alberta au Canada, et dans les comtés pratiquant l'élevage des cerfs dans le sud-ouest du Texas (États-Unis d'Amérique), pour décrire les divers problèmes rencontrés et les procédures épidémiologiques et prophylactiques utilisées. De nombreux progrès ont été réalisés dans la compréhension de la maladie, mais il est nécessaire de poursuivre les recherches sur le groupage génotypique des isolats du bacille du charbon, sur l'amélioration des techniques de diagnostic sur le terrain et sur les vaccins oraux, ainsi que sur les modalités de survie des spores dans le sol et sur l'écologie de la maladie dans des conditions naturelles.

Mots-clés

Afrique – Amérique du Nord – Bacillus anthracis – Diagnostic – Faune sauvage – Fièvre charbonneuse – Prophylaxie.

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Carbunco bacteridiano y fauna salvaje

M.E. Hugh-Jones & V. de Vos

Resumen
Aunque la incidencia del carbunco bacteridiano en el ganado esté disminuyendo en muchas partes del mundo, y seguramente haya cada vez más países realmente libres de la enfermedad, ésta es todavía enzoótica en muchos parques nacionales e incluso en zonas de cría de especies cinegéticas. Estas áreas infectadas pueden suponer un riesgo permanente no sólo para la salud pública sino también para el ganado de zonas aledañas, que de otro modo cabría declarar libres de la enfermedad. Sirviéndose de los ejemplos de los parques nacionales del Sur de África, el Wood Buffalo Park del Norte de Alberta (Canadá) y los condados de cría de cérvidos del sudoeste de Texas (Estados Unidos de América), los autores exponen la epidemiología de la enfermedad, los diversos problemas que ésta plantea y los procedimientos para controlarla. Aunque hoy se entiende y conoce mejor el carbunco bacteridiano, queda mucho por investigar sobre cuestiones tales como la clasificación genotípica de las muestras bacterianas salvajes, el perfeccionamiento de las vacunas orales y las técnicas de diagnóstico sobre el terreno o la adecuada comprensión de la supervivencia de las esporas en el suelo y la ecología de la enfermedad en condiciones naturales.

Palabras clave
África – Bacillus anthracis – Carbunco bacteriano – Control – Diagnóstico – Fauna salvaje – Norteamérica.

■

References

1. Akhmerov D.S., Kusov V.N. & Kusov A.A. (1982). – Survival of *Bacillus anthracis* in the tick *Dermacentor marginatus*. *Sbornik Nauchmykh Trudov, Kazanskii Veterinaryi Institut*, 101-103.
2. Almeyew H.S. (1936). – Die Darmform das Anthrax bei dem Geschlecht *Mustella* (Marder). *Dtsch. tierärztl. Wochenschr.*, **44**, 488.
3. Ambrosioni P. & Cremisini Z.E. (1948). – Epizoozia de carbonchio ematico negli animali del giardino zoologico di Roma. *Clin. vet.*, **71**, 143-151
4. Anon. (1959). – Anthrax at Glascow Zoo, Scotland. *In* International zoo yearbook (D.M. Morris, ed.). The Zoological Society of London, 52-53.
5. Anon. (1984). – Guidelines for vaccination of horses. Council Report. *J. Am. med. Assoc.*, **207** (4), 426-431.
6. Blancou J.-M. (1968). – Note clinique : cas de charbon bactéridien chez des carnivores sauvages de Madagascar. *Rev. Elev. Méd. vét. Pays trop.*, **21**, 339-340.
7. Böhm R. (1990). – Resistance, survival, sterilization and disinfection of spores of *Bacillus anthracis*. *Salisbury med. Bull.* (*Suppl.*), **68**, 99-101.
8. Braack L.E.O. & De Vos V. (1990). – Feeding habits and flight range of blow-flies (*Chrysomyia* spp.) in relation to anthrax transmission in the Kruger National Park, South Africa. *Onderstepoort J. vet. Res.*, **57**, 141-142.
9. Brennan A.D.J. (1953). – Anthrax with special reference to the recent outbreak in pigs. *Vet. Rec.*, **65**, 255-258.
10. Broughton E. (1987). – Diseases affecting bison. *In* Bison ecology in relation to agricultural development in the Slave River Lowlands NWT (H.W. Reynolds & A.W.L. Hawley, eds). Canadian Wildlife Service, Ottawa, 34-38.
11. Brunsdon J.R. (1968). – Unusual features in anthrax outbreaks. *Vet. Rec.*, **82**, 747-748.
12. Bryden H.B. & De Vos V. (1998). – Anthrax in the Kruger National Park, South Africa: the effect of different wild animals' sera on the *in vitro* germination of *Bacillus anthracis* spores. Preliminary report. *In* Proc. ARC-Onderstepoort OIE International Congress with WHO-Cosponsorship on anthrax, brucellosis, CBPP, clostridial and mycobacterial diseases, 9-15 August, Berg-en Dal, Kruger National Park. Sigma Press, Pretoria, 25-32.

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13. Buriro S.N. (1980). – Experimental inoculation of bacterial isolates obtained from *Argas (persicargas) persicus* in poultry. *Zeitschr. angew. Entomol.*, **89**, 324-330.

14. Buxton A. & Fraser G. (1977). – Bacillus. *In* Animal microbiology (A. Buxton & G. Fraser, eds). Blackwell Scientific Publications, Oxford, 492 pp.

15. Carpano M. (1932). – L'infezione da carbonchio ematico negli uccelli. *Bol. Ist. seiroter. milan.*, **11**, 161-173.

16. Carpano M. (1939). – Anthrax in seal. *Riv. milit. Med. vet. (Roma)*, **2**, 18-22.

17. Cartwright M.E., McChesney A.E. & Jones R.L. (1987). – Vaccination-related anthrax in three llamas. *J. Am. med. Assoc.*, **191**, 715-716.

18. Choquette L.P.E. & Broughton E. (1981). – Anthrax. *In* Infectious diseases of wild mammals, 2nd Ed. (J.W. Davis, L.H. Karstad & D.O. Trainer, eds). Iowa State University Press, Ames, Iowa.

19. Choquette L.P.E., Broughton E., Currier A.A., Cousineau J.G. & Novakowski N.S. (1972). – Parasites and diseases of bison in Canada. III. Anthrax outbreaks in the last decade in northern Canada and control measures. *Can. Field Naturalist*, **86**, 127-132.

20. Christoph H.-J., Reichel K. & Schnitzlein W. (1958). – Zum klinichen Bild des Milzbrandes bei Grosskratzen. *Kleintierpraxis*, **3**, 16-20.

21. Creel S., Creel N.M., Matovelo J.A., Mtambo M.M.A., Batamuzi E.K. & Cooper J.E. (1995). – The effects of anthrax on endangered African wild dogs (*Lycaon pictus*). *J. Zool. (London)*, **236**, 199–209.

22. De Vos V. (1974). – Vultures carry yet curtail anthrax. *J. S. Afr. vet. med. Assoc.*, **44**, 35.

23. De Vos V. (1990). – The ecology of anthrax in the Kruger National Park, South Africa. *Salisbury med. Bull. (Suppl.)*, **68**, 19-23.

24. De Vos V. (1998). – Anthrax in the Kruger National Park: an ecological approach to the question whether anthrax is indigenous to the Kruger National Park. *In* Proc. ARC-Onderstepoort OIE International Congress with WHO-Cosponsorship on anthrax, brucellosis, CBPP, clostridial and mycobacterial diseases, 9-15 August, Berg-en Dal, Kruger National Park. Sigma Press, Pretoria, 16-21.

25. De Vos V. (1998). – The isolation of viable and pathogenic *Bacillus anthracis* organisms from 200-year-old bone fragments from the Kruger National Park. *In* Proc. ARC-Onderstepoort OIE International Congress with WHO-Cosponsorship on anthrax, brucellosis, CBPP, clostridial and mycobacterial diseases, 9-15 August, Berg-en Dal, Kruger National Park. Sigma Press, Pretoria, 22-24.

26. De Vos V., Van Rooyen G.L. & Kloppers J.J. (1973). – Anthrax immunization of free-ranging roan antelope *Hippotragus equinus* in the Kruger National Park. *Koedoe*, **16**, 11-25.

27. De Vos V. & Bryden H.B. (1995). – The epidemiology of a major anthrax outbreak in the Kruger National Park. *In* Abstracts of the International Workshop on anthrax, 19-21 September, Winchester, United Kingdom, 25-26.

28. De Vos V. & Bryden H.B. (1996). – Anthrax in the Kruger National Park: temporal and spatial patterns of disease occurrence. *Salisbury med. Bull. (Suppl.)*, **87**, 26-30.

29. De Vos V. & Scheepers G.J. (1996). – Remote mass vaccination of large free-ranging wild animals for anthrax using Sterne spore vaccine. *Salisbury med. Bull. (Suppl.)*, **87**, 116-121.

30. De Vos V. & Bryden H.B. (1998). – The role of carnivores in the epidemiology of anthrax in the Kruger National Park. *In* Proc. Symposium on Lions and Leopards as Game Ranch Animals (J. Van Heerden, ed.), 24-25 October. SAVA Wildlife Group, Onderstepoort, Pretoria, 198-203.

31. De Vos V. & Bryden H.B. (1998). – Anthrax in the Kruger National Park: the role of roan (*Hippotragus equinus*) and sable (*H. niger*) in the epidemiology of anthrax. *In* Proc. ARC-Onderstepoort OIE International Congress with WHO-Cosponsorship on anthrax, brucellosis, CBPP, clostridial and mycobacterial diseases, 9-15 August, Berg-en Dal, Kruger National Park. Sigma Press, Pretoria, 33-36.

32. Dragon D.C. (2001). – Ecology of anthrax spores in northern Canada. PhD dissertation, University of Alberta, Edmonton, Alberta.

33. Dragon D.C. & Rennie R.P. (1995). – The ecology of anthrax spores: tough but not invincible. *Can. vet. J.*, **36**, 295-301.

34. Dragon D.C. & Elkin B.T. (2001). – An overview of early anthrax outbreaks in northern Canada. Field reports of the health of animals branch, Agriculture Canada, 1962-1971. *Arctic*, **54** (1), 32-40.

35. Dragon D., Mitchell J., Elkins B. & Coker P. (2001). – Anthrax in Wood Buffalo National Park, Alberta, 2000. 4th International Anthrax Conference, Annapolis, 10-13 June, Program abstracts, 13 pp.

36. Ebedes H. (1976). – Anthrax epizootics in Etosha National Park. *Madoqua*, **10**, 99-118.

37. Ebedes H. (1981). – A new look at anthrax. *In* Wildlife diseases of the Pacific Basin. Proc. Fourth International Conference of the Wildlife Disease Association, Sydney (M.W. Fowler, ed.). University of California, Davis.

38. Ferguson L.C. (1981). – Anthrax. *In* Diseases of swine, 5th Ed. (A.D. Leman, R.D. Glock, W.L. Mengeling, R.H.C. Penny, E. Scholl & B. Straw, eds). Iowa State University Press, Ames, Iowa.

39. Gainer R.S. (1987). – Epizootiology of anthrax and Nyasa wildebeest in the Selous Game Reserve, Tanzania. *J. Wildl. Dis.*, **23**, 175-178.

40. Gates C.C., Elkin B.T. & Dragon D.C. (1995). – Investigation, control and epizootiology of anthrax in a geographically isolated, free-roaming bison population in northern Canada. *Can. J. vet. Res.*, **59**, 256-264.

41. Gates C.C., Elkin B.T. & Dragon D.C. (1995). – Investigation, control and epizootiology of anthrax in a geographically isolated, free-roaming bison population in Northern Canada. *Can. vet. J.*, **59**, 256-264.

42. Gill I.J. (1982). – Antibiotic therapy in the control of an outbreak of anthrax in dairy cows. *Aust. vet. J.*, **58**, 214-215.

© OIE - 2002

43. Gleiser C.A. (1967). – Pathology of anthrax infection in animal hosts. *Fed. Proc.*, **26**, 1518-1521.

44. Graham-Smith G.S. (1913). – Flies and disease: non-blood sucking flies. Cambridge University Press, Cambridge.

45. Greenberg B. (1971). – Flies and disease, Vol. I. Princeton University Press, Princeton, New Jersey.

46. Greenberg B. (1973). – Flies and disease, Vol. II. Princeton University Press, Princeton, New Jersey.

47. Greener A.W. & Averil W. (1947). – Anthrax in mink (*Mustela vison*). *J. Hyg. (London)*, **39**, 149-153.

48. Hambleton P., Carman J.A. & Melling J. (1984). – Anthrax: the disease in relation to vaccines. *Vaccine*, **2**, 125-132.

49. Hanson R.P. (1959). – The earliest account of anthrax in man and animals in North America. *J. Am. med. Assoc.*, **135**, 463-465.

50. Harold Scot H. (1927). – An outbreak of anthrax at the zoological gardens. *Br. med. J.*, **1**, 229-230.

51. Harthoorn A.M. & Lock J.A. (1960). – A note on the prophylactic vaccination of wild animals. *Br. vet. J.*, **116**, 252-254.

52. Henning M.W. (1956). – Anthrax. *In* Animal diseases in South Africa, 3rd Ed. Central News Agency Ltd, South Africa.

53. Henning O. (1893). – Annual Report of the College of Veterinary Surgeons, C.G.H.

54. Henton M.M. & Briers G.F. (1998). – Anthrax spores isolated above burning carcases. *In* Proc. ARC-Onderstepoort OIE International Congress with WHO-Cosponsorship on anthrax, brucellosis, CBPP, clostridial and mycobacterial diseases, 9-15 August, Berg-en Dal, Kruger National Park. Sigma Press, Pretoria, 37-39.

55. Houston D.C. & Cooper J.E. (1975). – The digestive tract of the whiteback griffon vulture and its role in disease transmission among wild ungulates. *J. Wildl. Dis.*, **11**, 306-313.

56. Hugh-Jones M.E. (1999). – 1996-1997 Global report. *J. appl. Microbiol.*, **87**, 189-191.

57. Hugh-Jones M.E. & Hussaini S.N. (1974). – An anthrax outbreak in Berkshire. *Vet. Rec.*, **94**, 228-232.

58. Ikede B.O., Falade S. & Golding R.R. (1976). – Anthrax in captive carnivores in Ibadan, Nigeria. *J. Wildl. Dis.*, **12**, 130-132.

59. Jäger H.G., Booker H.H. & Hübschle O.J.B. (1990). – Anthrax in cheetahs (*Acinonyx jubatus*) in Namibia. *J. Wildl. Dis.*, **26**, 423-424.

60. Jordan W.J. (1964). – An outbreak of acute disease in Chester Zoo diagnosed as anthrax. *Vet. Rec.*, **76**, 927-930.

61. Kehoe D. (1917). – Anthrax in South Africa. *In* 5th and 6th Reports of the Director of Veterinary Education and Research, Union of South Africa.

62. Keim P., Kalif A., Schupp J., Hill K., Travis S.E., Richmond K., Adair D.M., Hugh-Jones M., Kuske C.R. & Jackson P. (1997). – Molecular evolution and diversity in *Bacillus anthracis* as detected by amplified fragment length polymorphism markers. *J. Bacteriol.*, **179**, 818-824.

63. Keim P., Price L.P., Klevytska A.M., Smith K.L., Schupp J.M., Okinaka R., Jackson P.J. & Hugh-Jones M.E. (2000). – Multiple-locus variable-number tandem repeat analysis reveals genetic relationships within *Bacillus anthracis*. *J. Bacteriol.*, **182** (10), 2928-2936.

64. Klemm D.M. & Klemm W.R. (1959). – A history of anthrax. *J. Am. med. Assoc.*, **135**, 458-462.

65. Knudson G.B. (1986). – Treatment of anthrax in man: history and current concepts. *Mil. Med.*, **151**, 71-77.

66. Kolonin G.V. (1970). – Evolution of anthrax. Report I. Peculiarities of circulation of the causative agent and the origin of the disease (English translation). *Zhurnal Mikrobiol. Epidemiol.*, **47**, 98-102.

67. Kolonin G.V. (1971). – Evolution of anthrax. Report II. History of spread of the disease (English translation). *Zhurnal Mikrobiol. Epidemiol*, **48**, 118-122.

68. Konrád J. Von (1967). – Klinische Differential sympto-matologie des Milzbrandes bei fleischfressenden Zootieren. *Kleintierpraxis*, **12**, 221-224.

69. Kriek N.P.J. & De Vos V. (1996). – Species differences in the pathology of wildlife in the Kruger National Park, South Africa. *Salisbury med. Bull. (Suppl.)*, **87**, 82.

70. Kronbergere H. von (1958). – Milzbrand bei Löwen. *Monatsh. VetMed.*, **13**, 235-237.

71. Lang E.M. (1964). – Milzbrand. *In* Verhandlungsberichte Internationales Symposium über die Erkrankungen der Zootiere, Wien, **6**, 49-50.

72. Lange L. (1901). – *Hyg. Rdsch.*, **11**, 529.

73. Leistner W. Von & Schuman E. (1956). – Milzbrand beim Löwen: Pathologisch-anatomische und veterinäpolizeiliche Betrachtungen. *Berl. Münch. tierärztl. Wochenschr.*, **69**, 425-427.

74. Lincoln R.E., Walker J.S., Klein F., Rosenwald A.J. & Jones W.I.J.R. (1967). – Value of field data for extrapolation in anthrax. *Fed. Proc.*, **26**, 1558-1562.

75. Lincoln R.E. & Fish D.C. (1970). – Anthrax toxin. *In* Microbial toxins (S.J. Ajl, S. Kadis & T.C. Montie, eds). Academic Press, New York.

76. Lindeque P.M. & Turnbull P.C.B. (1994). – Ecology and epidemiology of anthrax in the Etosha National Park, Namibia. *Onderstepoort J. vet. Res.*, **61**, 71-83.

77. Lindeque P.M., Brain C. & Turnbull P.C.B. (1996). – A review of anthrax in the Etosha National Park. *Salisbury med. Bull. (Suppl.)*, **87**, 24-26.

78. Lindeque P.M., Nowell K., Preisser T., Brain C. & Turnbull P.C.B. (1998). – Anthrax in wild cheetahs in the Etosha National Park, Namibia. *In* Proc. ARC-Onderstepoort OIE International Congress with WHO-Cosponsorship on anthrax, brucellosis, CBPP, clostridial and mycobacterial diseases, 9-15 August, Berg-en Dal, Kruger National Park. Sisgma Press, Pretoria, 9-15.

79. Lindsay D.R. & Scudder H.I. (1956). – Nonbiting flies and disease. *Annu. Rev. Entomol.*, **1**, 323-346.

© OIE - 2002

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

81. McConnell E.E., Tustin R.C. & De Vos V. (1972). – Anthrax in an African buffalo (*Syncerus caffer*) in the Kruger National Park. *J. S. Afr. vet. med. Assoc.*, **43**, 181-187.

82. McNary D.C. (1948). – Anthrax in American bison *Bos bison* L. *J. Am. med. Assoc.*, **112**, 378.

83. Miles J., Latter P.M., Smith I.R. & Heal O.W. (1988). – Ecological effects of killing *Bacillus anthracis* on Gruinard Island with formaldehyde. *Reclam. Reveget. Res.*, **6**, 271-283.

84. Minett F.C. (1952). – The annual and seasonal incidence of anthrax in various countries. Climatic effects and sources of infection. *Bull. Off. int. Epiz.*, **37**, 238-300.

85. Minett F.C. & Dhanda M.R. (1941). – Multiplication of *B. anthracis* and *B. chauvoei* in soil and water. *Indian J. vet. Sci. anim. Husb.*, **11**, 308-321.

86. Mitzmain M.B. (1914). – Insect transmission of anthrax. *J. trop. Med. Hyg.*, **17**, 61-62.

87. Moynihan W.A. (1963). – Anthrax in Canada. *Can. vet. J.*, **4**, 283-287.

88. Nishi J.S., Dragon D.C., Elkin B.T., Mitchell J., Ellsworth T.R. & Hugh-Jones M.E. (2001). – Emergency response planning for anthrax outbreaks in bison herds of northern Canada. *Ann. N.Y. Acad. Sci.* (in press).

89. Novakowski N.S., Cousineau J.G., Kolenosky G.B., Wilson G.S. & Choquette L.P.E. (1963). – Parasites and diseases of bison in Canada: II. Anthrax epizooty in the NorthWest Territories. *In* Transactions of the 28th North American Wildlife and Natural Resources Conference, 4-6 March, Washington, DC. Wildlife Management Institute, 233-239.

90. Office International des Epizooties (OIE) (1999). – Anthrax. *In* World Animal Health in 1999. Part 1: Reports on the animal health status and disease control methods and tables on incidence of List A diseases. Office International des Epizooties, Paris, 23.

91. Orr J.P., Johnston W.G. & Morrison J.R.A. (1978). – Anthrax lesions in a zoo cat. *Vet. Rec.*, **102**, 312-313.

92. Parry J.M., Turnbull P.C.B. & Gibson R.J. (1983). – A colour atlas of bacillus species. Wolfe Medical Publications Ltd, London, 272 pp.

93. Petrovski P. & Popev S. (1973). – Prilog kon pojavuvaneto na antraksot Kaj divite thivotni [Anthrax in Skopje zoo]. *Makedonski Veterinaren Pregled*, **2**, 61-65.

94. Pienaar U. de V. (1967). – Epidemiology of anthrax in wild animals and the control of anthrax epizootics in the Kruger National Park, South Africa. *Fed. Proc.*, **26**, 1496-1501.

95. Pienaar U. de V. (1973). – The capture and restraint of wild herbivores by mechanical methods. *In* The capture and care of wild animals (E. Young, ed.). Human and Rousseau, Cape Town and Pretoria, 91-99.

96. Plowright W. (1982). – The effects of rinderpest and rinderpest control on wildlife in Africa. *In* Animal diseases in relation to animal conservation (M.A. Edwards & U. McDonnell, eds). *Symp. zool. Soc. (London)*, **50**, 1-58.

97. Provost A. & Trouette M. (1957). – Réflexions sur quelques cas de charbon bactéridien (cryptique) chez des bovins. *Rev. Elev. Méd. vét. Pays trop.*, **10**, 25-26.

98. Rathore B.S. & Khera S.S. (1981). – Causes of mortality in felines in free-living state and captivity in India. *Indian vet. J.*, **58**, 271-276.

99. Redko S.A. (1926). – Le charbon bactéridien chez un ours. *Bull. Soc. Pathol. exot.*, **19**, 518-519.

100. Redmond C., Hall G.A., Green M. & Turnbull P.C.B. (1996). – Bacteriology, serology and pathology of experimental anthrax in pigs. *Salisbury med. Bull. (Suppl.)*, **87**, 79-82.

101. Robertson W. (1908). – Case of anthrax in an ostrich. *J. comp. Pathol. Therapeut.*, **21**, 361-362.

102. Schuaaf J. (1941). – Milzbrand bei Dachsen, Marden und Frettchen. *Tiehharztl. Rundsch.*, **43**, 514-515.

103. Seddon H.R. (1965). – Diseases of domestic animals in Australia. Bacterial diseases, Vol. I, Part V, 2nd Ed. Commonwealth of Australia, Department of Health.

104. Sen S.K. & Minett F.C. (1944). – Experiments on the transmission of anthrax through flies. *Indian J. vet. Sci. anim. Husb.*, **14**, 149-158.

105. Sen Gupta M.R. (1974). – A preliminary report on diseases and parasites of zoo animals, birds and reptiles. *Indian J. anim. Hlth*, **13**, 15-24.

106. Shrewsbury J.F.D. & Barson G.F.A. (1952). – A bacteriological study of the house sparrow, *Passer domesticus domesticus*. *J. Pathol. Bacteriol.*, **64**, 605-618.

107. Smith K.L., De Vos V., Bryden H.B., Hugh-Jones M.E., Klevytska A., Price L.B., Keim P. & Scholl D.T. (1999). – Meso-scale ecology of anthrax in southern Africa: a pilot study of diversity and clustering. *J. appl. Bacteriol.*, **87**, 204-207.

108. Smith K.L., De Vos V., Bryden H., Price L.B., Hugh-Jones M.E. & Keim P. (2000). – *Bacillus anthracis* diversity in the Kruger National Park. *J. clin. Microbiol.*, **36** (10), 3780-3784.

109. Smithcors J.F. (1957). – Evolution of the Veterinary Art. Kansas City, Missouri, Veterinary Medicine Publishing Co., 408 pp.

110. Sosnowski A. (1964). – Die Berkämpfung einer Milzbrandenzootie im Zoo Lódz. *In* International Symposium über die Erkrankungen der Zootiere, Wien, 51-53.

111. Stein C.D. (1945). – The history and distribution of anthrax in livestock in the USA. *Vet. Med. small Anim. Clin.*, **40**, 340-349.

112. Stein C.D (1948). – Incidence of anthrax in livestock during 1945, 1946 and 1947 with special reference to control measures in the various states. *Vet. Med.*, **43**, 463-469.

113. Stein C.D. (1955). – Anthrax. *Farmers Bull.*, **1736**, 1-14.

114. Sterne M. (1937). – The effects of different carbon dioxide concentrations on the growth of virulent anthrax strains. Pathogenicity and immunity tests on guinea pigs and sheep with anthrax variants derived from virulent strains. *Onderstepoort J. vet. Sci. anim. Ind.*, **9**, 49-67.

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115. Sterne M. (1937). – Variation in *Bacillus anthracis*. *Onderstepoort J. vet. Sci. anim. Ind.*, **8**, 271-349.

116. Sterne M. (1939). – The use of anthrax vaccines prepared avirulent (uncapsulated) variants of *Bacillus anthracis*. *Onderstepoort J. vet. Sci. anim. Ind.*, **13**, 307-312.

117. Sterne M. (1959). – Anthrax. *In* Infectious diseases of animals, Vol. I (A.W. Stableforth & I.A. Galloway, eds). Diseases due to Bacteria. Butterworths Scientific Publications, London, 16-52.

118. Sterne M. (1967). – Distribution and economic importance of anthrax. *Fed. Proc.*, **26**, 1493-1495.

119. Sterne M., Robinson E.M. & Nicol J. (1939). – The use of saponin spore vaccine for inoculation against anthrax in South Africa. *Onderstepoort J. vet. Sci. anim. Ind.*, **12**, 279-302.

120. Sterne M., Nicol J. & Lambrechts M.C. (1942). – The effect of large scale active immunization against anthrax. *J. S. Afr. vet. med. Assoc.*, **13**, 53–63.

121. Stiles G.W. (1944). – Isolation of the *Bacillus anthracis* from spinose ear ticks *Ornithodorus megnini*. *Am. J. vet. Res.*, **5**, 318-319.

122. Sussman A.S. & Halvorson H.O. (1966). – Spores, their dormancy and germination. Harper & Row, New York, 354 pp.

123. Theiler A. (1912). – Anthrax in the ostrich. *S. Afr. agric. J.*, **43**, 370-379.

124. Toschkoff A. & Veljanov D. (1970). – Sporulation und Virulenz von *Bacillus anthracis* in geöffneten und nicht geöffneten Tierleichen. *Arch. experim. VetMed.*, **24**, 1153-1160.

125. Turnbull P.C.B. (1986). – Thoroughly modern anthrax. Bureau of Hygiene and Tropical Disease. *Abstr. Hyg. trop. Dis.*, **61**, 1-13.

126. 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. *Vet. Rec.*, **128**, 399-403.

127. 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. *Epidemiol. Infect.*, **108**, 299-313.

128. Turnbull P.C.B., Bowen J. & Mann J. (1996). – Stubborn contamination with anthrax spores. *Environ. Hlth*, **104** (6), 171-173.

129. Turnbull P.C.B., Böhm R., Cosivi O., Doganay M., Hugh-Jones M.E., Joshu D.D., Lalitha M.K. & De Vos V. (1998). – Guidelines for the surveillance and control of anthrax in humans and animals, 3rd Ed. World Health Organization, Department of Communicable Disease Surveillance and Response, Document WHO/EMC/ZDI/98.6.

130. Turnbull P.C.B., Lindeque P.M., Le Roux J., Bennett A.M. & Parks S.R. (1998). – Airborne movement of anthrax spores from carcass sites in the Etosha National Park, Namibia. *J. appl. Microbiol.*, **84**, 667-676.

131. Turnbull P.C.B., Rijks J., Thompson I., Hugh-Jones M.E. & Elkin B. (2001). – Seroconversion in bison (*Bison bison*) in northwest Canada experiencing sporadic and epizootic anthrax. *In* 4th International Anthrax Conference, Annapolis, 10-13 June, Program abstracts, 18.

132. Ubertini B. (1939). – Un focolaio di infezione carbonchiosa ad insorgenza spontanea nelle anitre (*Chairina moschata*). *Clin. vet.*, **62**, 70-75.

133. Van Ness G.B. (1959). – Soil relationship in the Oklahoma-Kansas anthrax outbreak of 1957. *J. Soil Water Conserv.*, **14**, 70-71.

134. Van Ness G.B. (1971). – Ecology of anthrax. *Science*, **172**, 1303-1307.

135. Vasileva V.M. (1959). – A study of soil bacteria as antagonists of anthrax bacilli. *Sbornik Nanchmykh Trudov Lvovskii Zoovet. Inst.*, **9**, 149-153.

136. Verge J. & Placidi L. (1934). – La fièvre charbonneuse chez les animaux de ménagerie. *C.R. Séances Soc. Biol.*, **116**, 718-719.

137. Viljoen P.R., Curson H.H. & Fourie P.J.J. (1928). – Anthrax in South Africa. *In* 13th and 14th Reports of the Director of Veterinary Education and Research, Government Printing Office, Pretoria, 431-528.

138. Walker J.S., Klein F., Lincoln R.E. & Fernelius A.L. (1967). – A unique defense mechanism against anthrax demonstrated in dwarf swine. *J. Bacteriol.*, **93**, 2031-2032.

139. Whitford H.W. (1978). – Factors affecting the laboratory diagnosis of anthrax. *J. Am. med. Assoc.*, **173**, 1467-1469.

140. Whitford H. & Hugh-Jones M.E. (1994). – Anthrax. *In* Handbook of zoonoses, 2nd Ed. (G. Beran, ed.). Section A: bacterial, rickettsial and mycotic zoonoses. CRC Press, Boca Raton, Florida, 61-82.

141. Williams N. (1932). – Anthrax. *J. Am. med. Assoc.*, **81**, 9-25.

142. Zarubkinsky V.S. (1958/1959). – Self-purification of soil and water of *Bacillus anthracis* spores. *Sbornik Nanchnykh Trudov Lvovskii Zoovet. Inst.*, **9**, 51-58.

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