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# Tuberculosis in buffaloes (*Syncerus caffer*) in the Kruger National Park: Spread of the disease to other species

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**ABSTRACT**

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Tuberculosis, caused by *Mycobacterium bovis*, was recently diagnosed in a cheetah *(Acinonyx jubatus)*, two lions (*Panthera leo*) and a chacma baboon (*Papio ursinus*) from the Kruger National Park (KNP). It is assumed that they contracted the disease directly or indirectly from tuberculous buffaloes in the park. Tuberculous granulomatous lesions in the lungs were extensive and constituted the predominant changes in all three animal species. These pulmonary lesions included tuberculous bronchiolitis and cavitation which would facilitate dissemination of *M. bovis* into the environment. Spread of the disease to free-ranging species (in which it has not previously been reported) that may act as maintenance hosts of the infection, is a matter of serious concern.

**Keywords:** Chacma baboon, cheetah, Kruger National Park, lion, *Mycobacterium bovis*

## INTRODUCTION

Tuberculosis, caused by infection with *Mycobacterium bovis*, occurs widely in African buffaloes (*Syncerus caffer*) in the southern region of the Kruger National Park (KNP) (Keet, Kriek, Huchzermeyer & Bengis 1994; Bengis, Kriek, Keet, Raath, De Vos & Huchzermeyer 1996). In some buffalo herds the prevalence of tuberculosis is as high as 70 % (De Vos, unpublished data 1992). Although many surveys on diseases have been conducted in various mammalian species in the park over the years, tuberculosis caused by *M. bovis* has not been detected in animals other than buffaloes (Bengis *et al.* 1996). The diversity of free-ranging animal species within the borders of the park (for which it is renowned) (Pienaar, Joubert, Hall-Martin, De Graaf & Rautenbach 1987), is constantly in danger of contracting the disease because of the high levels of infection in certain regions of the park. This report documents the occurrence of tuberculosis in a cheetah *(Acinonyx jubatus)*, two lions (*Panthera leo*) and a chacma baboon (*Papio ursinus*) in the KNP, which they most likely contracted from tuberculous buffaloes.

## MATERIALS AND METHODS

### Animals

The cheetah was found dead in the veld and transported to the State Veterinarian's office at Skukuza. The two lionesses were immobilized in the veld, with a combination of zolazepam (250 mg) and tiletamine (250 mg) (Zoletil®, trademark: Logos Agvet, Private Bag X3, Halfway House, 1685) administered with a

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Dan-Inject® (trademark: Fritz Rohr, Private Bag X402, Skukuza, 1350) remote projector. While under anaesthesia, they were transported to Skukuza where they were euthanased (while still under anaesthesia) by the administration of an intravenous overdose of sodium pentobarbitone (Eutha-Nase®, trademark: Centaur, P.O. Box 912-686, Silverton, 0127) The chacma baboon was immobilized with Zoletil® (3 mg/kg) by means of a Dan-Inject® remote projector, but the baboon died within 4 min of being immobilized. Routine necropsies were performed on the lions and the baboon in the facilities of the State Veterinarian at Skukuza, KNP, within half an hour of euthanasia, and on the cheetah, immediately on arrival at Skukuza. It was estimated that the cheetah had been dead for about 2 h when the necropsy was started.

### Macro- and histopathology

Full necropsies were performed on the animals and all abnormalities recorded. Specimens of selected organs from all the animals were preserved in 10% buffered formalin, routinely processed and stained with haematoxylin and eosin for light-microscopic examination. Selected tissue sections and smears of the exudate were stained with the Ziehl-Neelsen stain to detect the presence of acid-fast bacteria.

### Bacterial isolation

Specimens of the lungs of the animals, as well as lymph nodes of one lion, and lymph nodes and the spleen of the baboon, were processed and cultured as described previously (Bengis *et al.* 1996).

### Sample preparation for PCR

Single mycobacterial colonies were picked from the media slopes and resuspended in 100 µℓ of sterile, double-distilled water. The suspensions were heated at 95 °C for 25 min.

### Oligonucleotide primers and amplification conditions

The PCR was performed in reaction volumes of 50 µℓ containing 40 pmol of each of the primers (TB-1A and TB-1B) (Cousins, Wilton, Francis, & Gow 1992), 1,5 mmol of $MgCl_2$, 50 mmol of KCl, 10 mmol of Tris-HCl, 0,1% Triton X-100, 50 nmol of each of the dNTPs and 1,5 units of Taq DNA polymerase (Promega: Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711-5399, USA). The reaction mixtures were overlaid with 50 µℓ of mineral oil and finally inoculated with 10 µℓ of heat-killed mycobacterial suspension.

Initial denaturation at 94 °C for 5 min, was followed by 30 cycles of temperature steps at 94 °C for 30 s, at 63 °C for 30 s and at 72 °C for 1 min. After a final extension step for 5 min, the samples were kept at 4 °C until analysed. Analysis of PCR results was performed on 3% agarose gels. Restriction enzyme digestion with *Sac* I was carried out on samples that showed successful amplification, to confirm the specificity of the amplification reaction (Cousins *et al.* 1992).

## RESULTS

### History and macroscopical pathology

The animals in which tuberculosis was diagnosed during the latter quarter of 1995, had been found in various regions of the park, where known TB-infected buffalo herds occur.

*Cheetah*

The carcase of a 2-year-old male cheetah was found in the Sabi Sand Wildtuin, which is part of the Greater KNP complex. The animal was emaciated, and manifested fairly extensive alopecia. At necropsy, the most significant lesions were found in the lungs. They contained numerous scattered granulomas, many of which contained liquefied, caseous necrotic exudate in their centres. Some lesions were cavitated. One, which occurred in the right caudal lobe, had ruptured, resulting in pneumothorax. Examination of a smear of the exudate, obtained from one lesion and stained with Ziehl-Neelsen stain, revealed many acid-fast bacilli.

*Lion*

Two lionesses suffering from tuberculosis were found, one at the Nwaswitshaka Water Hole, between Skukuza and Pretoriuskop in the south of the park, and the other, near the Satara Rest Camp in the central region of the park. Both lionesses were about 10 years old, had varying degrees of marked alopecia and an old, poorly-healed bite wound on the lumbosacral area. One animal was emaciated and could hardly stand when found, and had bilateral corneal opacity. Her lungs contained large areas of consolidation that became more obvious once the lungs had partially collapsed after the thoracic cavity had been opened. The lesions were ill-defined, sometimes confluent, firm, pliable foci about 4 cm in diameter. Many thin-walled cavities, containing small quantities of an opaque, mucoid exudate, occurred within the pneumonic areas. Very few acid-fast bacilli were detected in smears prepared from exudate obtained from these lesions.

The other lioness was in poor condition and depressed when captured. She had extensive lung lesions that occupied most of the tissue of both caudal lobes, and that were morphologically similar to those seen in the other lioness. When she was compared with the first lioness, however, many acid-fast

bacilli were present in the smears prepared from the exudate obtained from her lesions.

*Baboon*

The chacma baboon was an adult male that died shortly after having been immobilized in the Skukuza Rest Camp. He was in poor condition and depressed when found. At necropsy, his lungs were found to be affected by a severe, multifocal to confluent, granulomatous pneumonia that extended throughout all the lobes. There was variation in consistency and appearance on the cut surfaces in the individual granulomas. Some were fibrous, others were soft and contained large quantities of a yellowish-white, liquefied exudate, and a few of the smaller masses were calcified. Two large granulomas were also present in the spleen. Very large numbers of acid-fast bacilli were seen on smears prepared from the exudate.

## Histopathology

The major lesion in all three species, was granulomatous pneumonia which differed in essential features between the different animals. Multinucleated giant cells were prominent in the granulomatous reaction in the baboon, but were absent from the lesion in the felids. Extensive caseation of the exudate occurred in the cheetah and baboon, whereas no necrosis occurred in the inflammatory reaction in the lions.

*Cheetah*

Extensive areas of caseous necrosis occurred in the multifocal to coalescing granulomatous pneumonia. Multifocal pockets of neutrophils, occasional foci of coagulative necrosis, limited fibrin exudation, limited calcification, and occasional haemorrhage further characterized the process. Alveoli in the surrounding tissue were diffusely packed with macrophages and epithelioid cells. A few identifiable terminal bronchioles were plugged with necrotic exudate containing many neutrophils. Minimal fibrosis was evident at the periphery of the expansile granulomatous lesions. Acid-fast bacilli were sparsely distributed in the exudate and in the cytoplasm of macrophages at the periphery of the lesions.

Lymph nodes were atrophic, but showed no evidence of tuberculosis. Other organs were histologically normal.

*Lion*

Pneumonia in the lionesses consisted of an amorphous, multifocal to coalescing, expansile (non-encapsulated) granulomatous inflammatory reaction, without necrosis, giant cells or calcification. Haphazardly arranged macrophages and epithelioid cells in alveoli, formed areas of consolidation, interspersed with areas of acute inflammation characterized by fibrin and neutrophil exudation, and a marked inflammatory oedema. A mixed cellular exudate of macrophages and fibroblasts, and proliferating pneumocyte type-2 cells caused thickening of the alveolar walls. Alveoli outside the consolidated areas contained oedema fluid and many macrophages. Other features of lesser importance in the lungs included:

- focal areas of marked pulmonary oedema and emphysema
- thickening of the pleura due to a mild inflammatory reaction
- perivascular lymphoplasmacytic infiltrates
- occasional haemorrhage and erythrophagocytosis
- marked smooth-muscle hyperplasia in the walls of the terminal bronchioles.

A prolonged search did not reveal any acid-fast organisms.

Lymph nodes showed marked atrophy, but no evidence of tuberculosis. Extensive amyloidosis was evident in the medulla in both kidneys of one lioness. The medulla of the kidneys of the second lioness also contained deposits of amyloid, but to a much lesser extent.

*Baboon*

Areas of central caseation containing aggregates of necrotic neutrophils and, rarely, limited haemorrhage, were characteristic of the multifocal to confluent necrogranulomatous pneumonia seen in this animal. In these lesions, infiltrates of macrophages, epithelioid and multinucleated giant cells, and lesser numbers of lymphocytes and plasma cells, surrounded the necrotic debris. Although fibrous connective tissue was abundant, the lesions were non-encapsulated, and there was evidence of miliary spread. Liquefaction of central cores of the necrotic masses resulted in drainage of a purulent exudate into bronchioles. The bronchiolar epithelium had undergone focal caseous necrosis and ulceration. Limited calcification of the caseous necrotic debris was observed. Mild, multifocal, tuberculous granulomatous hepatitis and tuberculous granulomas, and extensive areas of caseous necrosis in lymph nodes and the spleen, that reflected systemic milliary spread, occurred. Many acid-fast bacilli were present in and around the necrotic tissue.

## Bacterial isolation and PCR analysis

*Mycobacterium bovis* was cultured from the specimens of all four animals and identified by standard methods. PCR amplification of all the mycobacterial isolates obtained from the three species produced a single, 372-bp DNA band, specific for the *M. tuberculosis* complex. In each case the band could be

enzymatically digested by *Sac* I rendering two bands, 220 bp and 152 bp in size, respectively.

## DISCUSSION

The diagnosis of tuberculosis, caused by *M. bovis,* in three additional species, confirmed spread of the disease in the KNP, to species other than buffaloes. This comes as no surprise, as it is known that the presence of just one maintenance host in an ecosystem with hardly any species diversity, results in spread of tuberculosis to other susceptible species within that system. In Great Britain, TB-infected badgers (*Meles meles*) are the cause of persistent, low-grade TB infection of cattle in the same ecosystem (Nolan & Wilesmith 1994) and, in New Zealand, tuberculous brushtail possums (*Richosurus vulpecula*) are the source of re-infection of cattle in the same environment (Coleman, Jackson, Cooke & Grueber 1994). Similarly, the presence of TB-infected ferrets (*Mustela putorius furo*) and feral cats in the MacKenzie Basin in New Zealand, is the reason for the persistent low-grade infection in cattle and deer in that area (Walker, Reid & Crews 1993).

The lions, in particular, most probably contracted the disease by consuming infected buffalo carcases, as it is known that, in zoos, they become infected when eating TB-infected meat (Eulenberger, Elze, Schuppel, Seifert, Ippen & Schroder 1992). Under free-ranging conditions in the KNP, a number of factors may increase the likelihood of lions contracting tuberculosis from TB-infected buffaloes. The intensity of predation by lions on buffaloes, is related to rainfall: increasing numbers of buffaloes which, up to the present time, were the only known infected species and source of the infection in the park, were caught by lions during years of prolonged drought (Mills, Biggs & Whyte 1995). This led to maximal predation of buffaloes by 1992, by which time the prevalence of tuberculosis in some of the buffalo herds was so high (up to 70%), that spill-over into the lion population became inevitable.

Inhalation of infective material is another way by which lions may become infected. Close contact while killing and feeding on the carcases of infected buffaloes shedding bacteria from open lesions, no doubt also constitute a source of infection, though this route is more likely to be of lesser importance. Once infected, dissemination of the infection within prides by droplet infection from tuberculous lions, is the most probable mechanism of transmission. This probability is enhanced by the localization of lesions in the lungs, and the presence of tuberculous bronchiolitis and cavitation that characterizes the lesions in this species. This method of transmission (both pseudo-horizontal and horizontal) is known to have caused disseminantion of the disease in groups of lions in zoos (Eulenberger *et al.* 1992). The gregarious lifestyle of lions, and their close association while socializing and feeding, also predispose to dissemination by inhalation of the infection, within prides. Finally, the high prevalence of feline immunodeficiency-virus infection in lions [a seroprevalence of 83% of lions in the KNP (Spencer, Van Dijk, Horzinek, Egberink, Bengis, Keet, Morikawa & Bishop 1992)], even though the infection is considered to be non-pathogenic in this species, may yet prove to increase the susceptibility of lions to *M. bovis*.

The manner in which the cheetah and baboon contracted the tuberculosis is uncertain. It is known that cheetahs are highly succeptible to tuberculosis and become infected by ingesting *M. bovis*-infected meat in zoos (Jones 1953) where they may die of the disease, on average, 2 years after having become infected (Van de Werken 1968). Although cheetahs are not commonly known to prey successfully on buffaloes or their offspring, nor to scavenge (Skinner & Smithers 1990), both events have been reported in the KNP (Pienaar 1969). Under present circumstances in the park, where large numbers of infected buffaloes occur, it is possible that the infected cheetah contracted the disease by consuming the organs of a tuberculous buffalo.

The occurrence of tuberculosis caused by *M. bovis* has only once been reported in free-living baboons in Africa (Tarara, Suleman, Sapolsky, Wabomba & Else 1985). In this instance, the source of the infection was discarded *M. bovis*-infected organs of cattle slaughtered at the local village abattoir (Sapolsky & Else 1987). Baboons are omnivores, and may also have contracted the disease in the KNP by eating infected buffalo organs. The possibility, however, of yet another infected host, such as a small mammal [for instance hares (*Lepus* sp.)] that have been shown to be naturally infected in South Africa (Paine & Martinaglia 1928) and may be eaten by both cheetah and baboon, should be considered as another, and possibly more likely, source of the infection.

Although the survival period for mycobacteria in the environment has not been determined in South Africa, environmental contamination appears to be a less likely source of infection in the KNP, as most of the mycobacteria are rapidly destroyed when exposed to the harsh African climate (Morris, Pfeiffer & Jackson 1994). They are not, however, eliminated at such a rate that they do not constitute a hazard to other species, as the rate of disappearance from the environment is measured in days or weeks rather than hours. Such bacteria may be found in exudates, faeces, and carcase material that has contaminated soil, water, grass or plants (O'Reilly & Daborn 1995).

It is expected in both cheetahs and baboons (as is the case in lions) that, once the infection has established itself in those populations, further spread of the disease may occur by droplet infection. Although

this is expected to occur easily in baboons because of their gregarious way of living, transmission from infected baboons to others in their troop did not occur in the one outbreak of tuberculosis caused by *M. bovis* reported in the literature (Sapolsky & Else 1987). In cheetah, although they occur in small groups, males form permanent coalitions, and there is close contact between males and females for a number of days while they are breeding, thus enhancing the possibility that spread can occur by inhalation.

The diagnosis of tuberculosis in other species in the KNP reported in this paper, is a serious matter. This paper records an overwhelming infection detected in various animals in a naive and fully susceptible population. Because of their way of living, there is every reason to believe that the infection will become established in these species. If this is the case, they will become maintenance hosts of the disease. This has serious implications for control measures against the disease in the park, since, once this infection is established in more than one species, no matter how low the prevalence of the disease, eradication becomes virtually impossible, as these maintenance hosts will perpetuate the infection. The presence of such a large number of maintenance hosts in the diverse ecosystem of the KNP may be expected to infect further potential maintenance or spill-over hosts (Grange & Collins 1987), and further spread of the disease to other species in the park and neighbouring areas becomes a certainty. Additionally, the close association between humans and baboons in the park, particularly in some of the tourist camps and local dwellings, should be a cause for concern, as the infection, which is a known zoonosis, may also be contracted by man.

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