



Tuberculosis in kudu (*Tragelaphus strepsiceros*) in the Kruger National Park

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ABSTRACT

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Five kudu (*Tragelaphus strepsiceros*), three bulls and two cows, within the Greater Kruger National Park complex, were diagnosed with generalized tuberculosis caused by *Mycobacterium bovis*. The lesions seen in these animals were similar to those previously reported in kudu and included severe tuberculous lymphadenitis of the nodes of the head and neck (that resulted in noticeable uni- or bilateral swelling beneath the ear), thorax, and the mesentery. All the animals also suffered from severe granulomatous pneumonia. The lesions in the lungs were more severe cranially and had a miliary distribution elsewhere in the lungs. Based on the DNA patterns of the *M. bovis* isolates, at least some of these kudus were infected with strains commonly present in tuberculous buffaloes, lions, cheetahs, and baboons in the Park whereas other strains from these kudus were quite different and may reflect another source of infection. The presence of tuberculous kudu in the Park is expected to complicate control measures that may be instituted to contain or eradicate the disease in the Park.

Keywords: Buffalo, kudu, *Mycobacterium bovis*, *Syncerus caffer*, *Tragelaphus strepsiceros*, tuberculosis

INTRODUCTION

In the Kruger National Park (KNP), tuberculosis, caused by *Mycobacterium bovis*, was first reported in 1990 in a moribund buffalo bull (*Syncerus caffer*) found close to the southern border of the Park (Bengis, Kriek, Keet, Raath, De Vos & Huchzermeyer 1996). It was subsequently determined that the infection was widespread with the highest prevalence in buffalo herds in the southern regions of the Park (Bengis *et al.* 1996). Tuberculosis (caused by *M.*

bovis) considered to be due to a spillover from the infected buffaloes, has subsequently been reported in lions (*Panthera leo*), cheetahs (*Acinonyx jubatus*) and baboons (*Papio ursinus*) in the Park (Keet, Kriek, Penrith, Michel & Huchzermeyer 1996).

Because of the very high prevalence of the infection in some of the buffalo herds, estimated to be as high as 90 %, spillover into additional species was expected. As it has previously been reported that free-ranging kudu were infected with *M. bovis* after contact with tuberculous cattle (Paine & Martignalia 1928; Thorburn & Thomas 1940), and remain so to this day (Weber & Van Hoven 1992) in the Eastern Cape Province of South Africa, it was not unexpected that, eventually, kudu would also become infected in the KNP. Tuberculosis in kudu may be subclinical but frequently is characterized by the development of large, uni- or bilateral swellings in the parotid region in the advanced stage of the disease. This feature allows detection of tuberculous animals from a distance (Thorburn & Thomas 1940).

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This communication reports the presence of tuberculosis caused by *M. bovis* in kudu in areas of the greater KNP complex where tuberculous buffaloes are present.

MATERIALS AND METHODS

Detection of infected kudu

Inspectors of the Directorate of Animal Health and game rangers were involved in detecting infected kudu that were emaciated or cachectic, or were recognizable by virtue of the presence in the parotid area of large, uni- or bilateral swellings caused by tuberculous granulomas in the regional lymph nodes (Thorburn & Thomas 1940) (Table 1). One old bull was blind. Affected animals were killed by shooting them through their neck with a high-powered rifle. Thereafter the dead animals were conveyed by road to Skukuza Camp where a post mortal examination was performed in the necropsy facilities of the local state veterinarian. The interim, from the time that they were shot until the commencement of the necropsies, did not exceed 6 h.

Necropsies

Routine, complete necropsies were performed and the lesions observed were recorded. Smears of the exudate of lesions were prepared and specimens for bacterial culture and histopathological examination were collected during the procedure.

Smears

The smears were air-dried, fixed in 70% ethanol, stained with the Ziehl-Neelsen acid-fast stain, and examined under a light microscope for the presence of acid-fast bacteria.

Histopathology

Specimens of affected tissues were fixed in 10% buffered formalin. Tissue blocks were cut from these specimens (after they had been fixed in the formalin for at least 48 h) and processed using routine methods for light microscopy. The tissue sections were stained with haematoxylin and eosin, and sections from selected blocks with the Ziehl-Neelsen stain for the detection of acid-fast bacteria.

Bacterial isolation

Specimens for culture were collected from lesions in the lung and from the lymph nodes of the head, thorax or mesentery. In two cases, samples of the fibrous wall and fluid contents present in the fluctuating swelling below the ears were also collected. The specimens were placed individually in sterile containers, frozen at -20°C , and submitted to the Onderstepoort Veterinary Institute where they were cultured and identified according to the methods of Bengis *et al.* (1996).

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Sample preparation and PCR amplification

Heat-killed mycobacterial suspensions were prepared and amplified as has been described by Keet *et al.* 1996.

Genomic typing by RFLP

Mycobacterium bovis isolates were grown in 7H9 Middlebrook broth for 4–6 weeks and then heat-inactivated at 80°C for 25 min. DNA extraction, enzymatic digestion with *Pvu II* and agarose gel electrophoresis with subsequent Southern blotting were carried out as described by Skuce Brittain, Hughes, Beck & Neill (1992).

RFLP probe and hybridization

The entire IS 6110 sequence was amplified (Skuce *et al.* 1992) by PCR with simultaneous non-radioactive labelling with DIG (Boehringer Mannheim, Sandhofer Strasse, 68298 Mannheim, Germany) and used as a probe during hybridization. For the post-hybridization washes and the enzymatic detection of the DIG-labelled hybridization product the instructions of the manufacturer were followed.

Genomic typing of *M. bovis* isolated from the various specimens was performed by previously described methods.

RESULTS

Clinical signs

Eighteen kudu with uni- or bilateral swelling of the parotid area were reported within the KNP from October 1995 to September 1996. Of these, five (three bulls and two cows) were shot for the investigation (Table 1). The condition of the animals varied from good to emaciated. One of the two cows was lactating and the other one was pregnant. Bulging, fluctuant swellings were present beneath one or both ears in three of the five animals, some extending up to 400 mm ventral to the ear. In four of the animals, at least one fistulous tract was present in the swollen area. A needle aspiration of the contents of the swellings usually produced turbid, pale, yellowish, very watery pus.

Macroscopic pathology

The lesions seen in the five animals varied in severity but the disease was generalized in all of them, being most advanced in the bulls. All the lymph nodes of the head, neck and thoracic cavity, as well as some of the pre-scapular and mesenteric; and the hepatic

TABLE 1 Tuberculous kudu in the Kruger National Park and Sabi-Sand Game Reserve

Kudu no.	Locality	Age and clinical signs	Condition
1	Mbyamithi camp	Old male; blind; large fluctuating swelling with draining fistula beneath left ear	Cachectic
2	Mbyamithi weir	Old male	Emaciated
3	Sabi Sand GR ¹	Adult female; bilateral, moderate, parotid swellings	Good; lactating
4	Sabi Sand GR	Adult female; bilateral, moderate, parotid swellings	Good; pregnant
5	Mbyamithi camp	Adult male	Emaciated

¹ Sabi Sand Game Reserve

nodes were affected in these animals. Affected nodes were irregularly enlarged, encapsulated, firm, and reflected a mixed granulomatous and caseous necrotic appearance on cut surface. The bronchial lymph nodes were markedly enlarged (up to 100 mm in diameter) and their normal structure was completely replaced by a severe, granulomatous inflammatory reaction that also contained large masses of caseo-necrotic debris. Lymphatics draining into a lymph node were often affected by multifocal, granulomatous lymphangitis, the individual granulomas in the lymphatics being up to 2 mm in diameter.

The swellings in the parotid area, when cut into, revealed cavities filled with a watery, turbid, whitish exudate enveloped by a thin, well-vascularized capsule. The pressure exerted by large volumes of accumulated exudate caused the fluid to dissect between the anatomic structures of the neck. Ventral to the ears (where the parotid salivary gland and lymph nodes are situated), large, poorly-encapsulated, caseo-necrotic masses replaced the normal tissue.

The lungs of all three bulls were severely diseased, the extent and nature of the granulomas varying according to their locality. Those in the cranial lobes were larger and more numerous than in the caudal lobes, and replaced most of the normal parenchyma causing extensive areas of consolidation. The granulomas in the cranial lobes were crowded together, even-sized (30–40 mm in diameter), ovoid, oblong or triangular, and well-encapsulated. They contained a caseo-necrotic exudate that was smooth and inspissated, sometimes partially liquefied, and poorly calcified. Similar granulomas occurred in the caudal portions of the lungs but as few isolated masses scattered randomly throughout the lobes. In addition, miliary tubercles appearing as pearly areas of granulomatous inflammation that did not exceed about 3–5 mm in diameter were scattered throughout the caudal lobes. These small granulomas tended to be clustered together imparting a mulberry-like appearance to the lesion. The diffuse granulomatous pleuritis that predominantly involved the affected portions of the cranial lobes resulted in fibrous adhesions to the parietal pleura.

In one of the bulls, tuberculous enteritis was also detected and another had scattered tuberculous lesions in the liver and renal cortex. The granulomas in these organs were small, roundish, about 2 mm in diameter but were not encapsulated.

Corneal opacity and a fibrinous exudate in the anterior chamber of both eyes were detected in the blind bull.

The two cows were less severely affected and lesions were predominantly present in the lymph nodes of the head. These nodes were not as enlarged as were the nodes in the bulls. The nodes of the neck were less often involved though a granulomatous lymphadenitis of one prescapular node was seen in one of the cows, and of a hepatic node in the other. Both had a multifocal, granulomatous, mesenteric lymphadenitis. The lesions in the lungs of these two animals were limited to a few caseo-necrotic granulomas scattered throughout the parenchyma of the lung.

Histopathology

The lesions present in the animals that were necropsied were similar on histopathological examination. They were encapsulated and contained extensive areas of caseous necrotic debris surrounded by a typical granulomatous inflammatory reaction characterized by the presence of numerous epithelioid cells, Langhans' giant cells, and lymphocytes. The lesions were further characterized by the presence of large numbers of neutrophils particularly in the caseous necrotic debris. Even very early lesions contained aggregates of neutrophils. Few acid-fast bacteria were present in the cytoplasm of Langhans' giant cells and in the exudate.

Bacterial isolation

Mycobacterium bovis infection was confirmed in all five kudu by standard culture and biochemical identification. The bacterium was successfully isolated from the lungs of all five kudus, both samples of the exudate and fibrous wall of the lesion, and the parotid, bronchial and mesenteric lymph nodes.

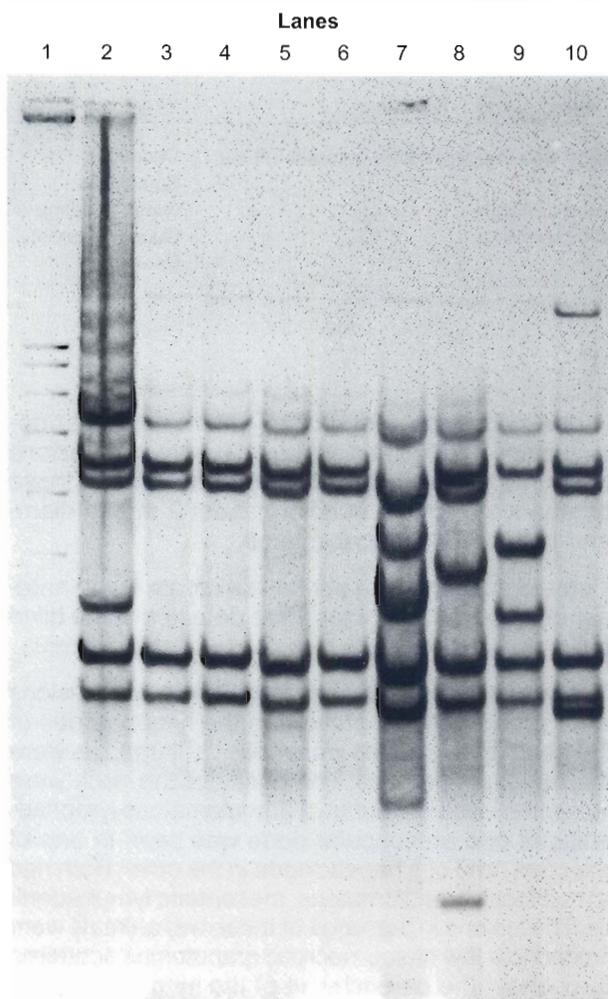


FIG. 1 DNA from *M. bovis* isolates digested with *Pvu*II and hybridized with DIG-labelled IS6110 (left to right)

- Lane 1: molecular weight marker VII_a
- Lanes 2–6 and 9–10: *M. bovis* isolates from different buffaloes from the KNP
- Lane 7: Kudu 1 from the KNP
- Lane 8: Kudu 2 from the KNP

PCR analysis

Amplification of all *M. bovis* isolates by PCR resulted in the production of a single DNA band consistent in size with the 372 bp band described by Cousins, Wilton, Francis & Gow (1992). The specificity of the reaction was confirmed by enzymatic digestion with *Sac* I that rendered two distinct bands of 220 bp and 152 bp, respectively.

RFLP analysis

Mycobacterium bovis isolates from four of the five kudus were typed using the entire *IS6110* and *IS1081* sequences as probes.

TABLE 2 DNA patterns obtained from the *M. bovis* isolates from kudu

Animal no.	DNA fingerprint	
	IS6110	IS1081
1	9	1
2	1	1
3	1	1
4	9	1

RFLP analysis using IS1081

A common DNA pattern designated IS-1 was observed in all four isolates.

RFLP analysis using IS6110

Two distinct RFLP types were identified; both consisted of 12 bands referring to six copies of *IS6110* (Fig. 1). The patterns for kudu 1 and 4 were similar (IS-9) and those of kudu 2 and 3 (IS-1) were similar (Table 2).

DISCUSSION

This report confirms the presence of *M. bovis*-infected kudu within the borders of the greater KNP complex in areas where known TB-infected buffaloes occur. The lesions and their distribution in these tuberculous kudu resemble those described in kudu in the Eastern Cape Province by Thorburn & Thomas (1940) and Weber & Van Hoven (1992).

Based on the results of the DNA typing of the isolates of *M. bovis* obtained from these animals, it appears that, at least, some of them were infected with the common type of *M. bovis* (IS-1) present in infected buffaloes in the Park (A. Michel, unpublished data 1996). This is also the type isolated from lions, cheetahs, and baboons in the Park. To date this type (IS-1) has not been found in any other species outside the Park with the exception of tuberculous commercial cattle bordering the south of the Kruger National Park (Michel, unpublished data 1996). From these data, it appears that kudu in all likelihood contracted the disease because of spillover of the infection from high prevalence buffalo herds and the existence of epidemiological links between the infected species in the Park. However, since a strain (IS-9) totally different from those isolated from buffaloes in the Park were isolated from two kudus (kudu 2 and 3), it appears that yet another, unidentified, source of the infection may exist in the KNP or in its surrounding areas, as a similar "foreign" type of *M. bovis* was isolated from a kudu shot on an adjacent farm in the Malelane area on the southern border of the KNP (Bengis, Keet, Michel & Kriek 2001).

Following detection of tuberculosis in yet another species in the Park, one can only reflect on the prophetic words of Martinaglia published in 1930: "As a possible menace to the fauna of our national game reserves, tuberculosis must be considered. The advice of a veterinary epizootologist should be constantly available to those in charge of the reserves, in order to control the introduction of infectious diseases." This, despite the statements [as quoted by Thorburn & Thomas (1940)] by Calmette & Fox, respectively, that "Wild animals ... never contract tuberculosis spontaneously", and that "there are no reliable data concerning the existence of tuberculosis in the wild".

The presence of tuberculous kudus in the Park should be viewed with concern. Not only may these animals play a role in maintaining and disseminating tuberculosis, but also, because of the ease with which kudus cross game fences, they may disseminate the infection to commercial and communal stock, and game on farms close to the border of the Park.

The role of kudus as a maintenance host of tuberculosis is largely unknown. However, it is clear that the increase in prevalence of tuberculosis in kudus at the turn of the century was related to the increase in numbers of these animals in the Eastern Cape Province following their protection by an act of Parliament (Thorburn & Paine 1940). It is uncertain as to whether the increased prevalence of tuberculosis in kudu reflected a similar increase of the disease in cattle. Judging by the few tuberculous cattle detected at abattoirs in the area at the time (less than one out of 17 263 cattle slaughtered over a period of 5.5 years), it does not appear to be the case, although there is some doubt whether farmers sent known TB-infected cattle for slaughter to abattoirs (Thorburn & Thomas 1940).

The current knowledge about the disease in kudus implies that, despite declining infection rates of tuberculosis in domesticated cattle in South Africa, the disease, be it at low levels, appears still to be present in kudus in the Eastern Cape Province after all these years. As recently as 1992, Weber & Van Hoven reported the presence of a tuberculous kudu among ten, randomly culled kudus from the Addo Elephant National Park in the Eastern Cape Province. It may be that, once infected, the number of kudus in a specific area is more important for maintaining the disease, than the presence of tuberculous cattle.

The extent of the threat of infected kudus is placed in perspective by the following discussion by Thorburn & Thomas (1940): "It will be appreciated that the kudu not only constitutes an additional susceptible animal, but that it also acts as an active disseminator of virulent material both by reason of its habits and in the peculiar way in which it reacts to the disease. In other words, the kudu becomes an open case of

tuberculosis (discharging glands) long before the disease is generalized, and that when it becomes generalized, the lung, intestine and other excretory organs merely become additional routes for the dissemination of the bacilli. One infected kudu is a far greater danger than an infected bovine, because: (a) it discharges infective material over long periods, and (b) it knows no farm boundaries and may carry infection to one or more clean farms perhaps miles away from the original source."

Considering the above factors, it is clear that the presence of tuberculous kudus introduces a further dimension to the difficult task of trying to control tuberculosis in the KNP and its surroundings. Not only is it likely that kudus may act as maintenance hosts in the Park and surrounding farming areas, but they also appear to reflect the presence of a source of infection different from that of buffaloes in the Park.

The fact that kudus are also now known to be infected with *M. bovis* should be taken cognisance of in planning and implementing control strategies for tuberculosis in the Park and surrounding farming areas.

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