

An outbreak of bovine tuberculosis in a free-living African buffalo (*Syncerus caffer*—Sparrman) population in the Kruger National Park: a preliminary report

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ABSTRACT

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Bovine tuberculosis was diagnosed for the first time in an African buffalo (*Syncerus caffer*) in the Kruger National Park (KNP). The index case was a 2-year-old, emaciated bull which had been found recumbent and obviously ill, near the south-western boundary of the KNP, in July 1990. During a follow-up random sampling of 57 buffalo, from two herds in close proximity to this initial case, nine more suspect cases were found. *Mycobacterium bovis* was isolated from a lung and thoracic lymph node, respectively, of two of these cases. Histopathologically, all nine of these animals had granulomatous lesions compatible with a diagnosis of mycobacteriosis, but acid-fast organisms could be demonstrated in only one animal.

Keywords: African buffalo, bovine tuberculosis, Kruger National Park, *Mycobacterium bovis*, *Syncerus caffer*

INTRODUCTION

Bovine tuberculosis has frequently been described in wild animals, particularly those in zoological gardens (Montali 1978). In captive animals in Africa, bovine tuberculosis has been described in springbok (*Antidorcas marsupialis*) (Robinson 1953; Hofmeyr

1956), giraffe (*Giraffa camelopardalis*) (Martinaglia 1930; Basson, McCully, Kruger, Van Niekerk, Young & De Vos 1970); black rhinoceros (*Diceros bicornis*), African buffalo (*Syncerus caffer*) and nyala (*Tragelaphus angasii*) (Hofmeyr 1956).

Tuberculosis caused by *M. bovis* has also been reported in free-living wildlife populations in Africa. Paine & Martinaglia (1928) reported the first cases in Cape (greater) kudu (*Tragelaphus strepsiceros*) and common duiker (*Sylvicapra grimmia*) in South Africa. Tuberculosis, also caused by *M. bovis*, occurred endemically in greater kudu in the eastern Cape region of South Africa (Thorburn & Thomas 1940; Robinson 1944). A case of mycobacteriosis in a free-ranging black rhinoceros in the Hluhluwe Game Reserve in Kwazulu-Natal was reported by Keep & Basson (1973). Pulmonary tuberculosis caused by *M. bovis* has also been reported in free-living lechwe

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antelope (*Kobus lechwe*) in Zambia (Gallagher, Macadam, Sayer & Van Lavieren 1972; Clancey 1977).

Bovine tuberculosis in free-living African buffalo was first described in the Queen Elizabeth National Park in Uganda (Guilbride, Rollison, McAnulty, Alley & Wells 1963; Thurlbeck, Butas, Manciewicz & Laws 1965. Woodford (1982a and b) speculated that tuberculous cattle from areas bordering the Park, may have been the source of infection, since it was known that buffalo and cattle sometimes shared grazing areas. He estimated that bovine tuberculosis was responsible for an annual mortality of 1% of the 18 000 buffalo in the park. He also found that warthog (*Phacochoerus aethiopicus*) was the only other species endemically infected in that national park.

In the Kruger National Park (KNP), mycobacteriosis was first recorded in November 1967 in an impala (*Aepyceros melampus*) from the Crocodile Bridge area (De Vos, McCulley & Van Niekerk 1977). In this isolated case, no bacterial isolation was attempted, but the histopathological features (numerous acid-fast organisms present) suggested a diagnosis of atypical or avian mycobacteriosis.

A previous intensive survey (1966) into the diseases of buffalo in the southern region of the KNP (Basson *et al.* 1970), involving 100 complete necropsies, failed to detect any mycobacterial infection, although granulomatous lymphadenitis of unknown aetiology was found in two animals. Granulomatous lesions in the lymph nodes of buffalo were common in this survey, but all except the above-mentioned two specimens were of mycotic aetiology.

Furthermore, between 1964 and 1974, the carcasses of 11 985 impala, 2 067 blue wildebeest, 8 701 buffalo and 5 031 elephant were intensively inspected during routine meat inspection at the Skukuza game abattoir. No lesions suggestive of tuberculosis were recorded.

This report deals with an outbreak of tuberculosis in buffalo in the south-western corner of the KNP. It was first detected in 1990, after a buffalo was found moribund, and euthanased for necropsy.

MATERIALS AND METHODS

The initial case of tuberculosis was diagnosed during an opportunistic necropsy performed on an emaciated, moribund 2-year-old buffalo bull reported by the local game ranger near the western boundary fence in the southern region of the KNP. After the histopathological confirmation of mycobacteriosis, a survey was launched to sample the two buffalo herds closest to where this case had been found. A total of 57 animals were culled out of these two herds, by means of the standard KNP buffalo-culling technique, (De Vos, Bengis & Coetzee 1983).

Necropsy procedures

Standard necropsy procedures were combined with routine meat inspection. After the buffalo had been killed, they were eviscerated in the field, and a detailed examination of the abdominal viscera was carried out *in situ*, with particular attention being paid to the mesenteric and other intra-abdominal lymph nodes. The thoracic organs were left in the carcass, and removed for examination only after arrival at the abattoir. At the abattoir, the carcasses were further processed and inspected according to standard meat-inspection procedures. Particular attention was paid to the lungs which were thoroughly palpated and incised, as were the bronchial and mediastinal lymph nodes, as well as the cranial lymph nodes and the palatine tonsils.

Histopathology

Specimens were collected and preserved in 10% buffered formalin, and were later prepared routinely for light microscopy by embedding them in paraffin wax. Sections cut to a thickness of 4–6 µm were routinely stained with haematoxylin and eosin, and with Ziehl-Neelsen acid-fast stain.

Bacteriology

Methods for isolation and identification of the bacterium have been described (Anon. 1983, Nel, Kleeberg & Gatner 1980). Lesions were excised and homogenized. The homogenized tissue was divided into two parts, one part being decontaminated with 2% HCl, and the other with 4% NaOH. The samples were left at room temperature for 15 min, then centrifuged for 15 min at 1 650 g. The supernatant fluid was discarded, and the sediments washed by centrifugation, then inoculated onto each of two tubes of:

- Löwenstein-Jensen (LJ) egg-based medium with glycerine
- LJ medium without glycerine
- LJ medium with 0.5% pyruvate

The tubes were incubated at 37°C and observed for growth of colonies of acid-fast bacteria at 1 and 2 weeks, and then at 2-weekly intervals for 10 weeks. As soon as colonies of acid-fast bacteria were observed, the isolates were subcultured onto the same medium, which supported primary growth. The isolates were then identified by:

- their growth characteristics
- their preference for LJ medium supplemented with pyruvate
- their sensitivity to thiophene carboxylic-acid-hydrazide
- the results of biochemical tests

RESULTS

The initial case was euthanased in the field in the late afternoon and the necropsy was performed with the aid of the headlights of a vehicle, owing to poor lighting conditions. The lungs were riddled with disseminated, firm nodules varying in size from 5–110 mm in diameter. On cut section, these nodules were whitish grey with varying numbers of small (2–4 mm) necrotic, yellowish foci distributed haphazardly within the nodule, characteristic of a granulomatous inflammatory reaction. No pleural lesions were present. The bronchial and mediastinal lymph nodes were all grossly enlarged (50–110 mm in diameter), owing to the presence of a similar granulomatous reaction. No similar lesions were observed in any other visceral organ. The only other macroscopic pathological finding was that of severe cachexia.

At the follow-up survey cull involving 57 buffalo, three more cases with similar, but less advanced, typical macroscopic granulomatous lesions involving both the lungs and thoracic lymph nodes were identified. A further six buffalo had suspicious granulomatous lesions in the lungs or in one or more lymph nodes. Microscopically, the lesions from all nine animals were characterized by a pronounced granulomatous reaction containing accumulations of necrotic lymphocytes surrounded by variable numbers of Langhans' giant cells, epithelioid cells and lymphocytes. Some lesions were well encapsulated, and others showed varying degrees of caseation. In only one specimen could sections of acid-fast organisms be demonstrated.

A *Mycobacterium* was cultured from samples of two buffalo sent for bacterial isolation. Small numbers of colonies of acid-fast bacteria (range 3–18 colonies/buffalo sample) grew on LJ medium supplemented with pyruvate after 4 weeks' incubation at 37°C. No growth was obtained on LJ medium, either with or without glycerine. Growth characteristics and results of biochemical tests as listed in Table 1, identified the isolates as *M. bovis*.

Finally, a few acid-fast rods were also seen microscopically in Ziehl-Neelsen-stained smears of the

lung of one of these culture-positive buffalo, but no acid-fast rods were found in smears of the lymph node of the other culture-positive buffalo.

DISCUSSION

For the first time, bovine tuberculosis has been positively diagnosed in free-living buffalo in the KNP. It would appear that bovine tuberculosis has only relatively recently entered the KNP. The fact that a previous intensive survey of the diseases of buffalo in the southern area of the KNP (Basson *et al.* 1970), and routine meat inspection of over 8000 buffalo carcasses at the Skukuza Game Abattoir between 1970 and 1980 failed to positively detect the disease, supports the premise. However, in this buffalo-survey cull carried out in 1990, 9/57 or 15.8% animals had lesions compatible with those of tuberculosis, indicating that this disease had been present in those herds for several years or decades. The probable reasons why the disease remained undetected for such a period of time are:

- An initial low prevalence rate in recently infected herds
- Between 1980 and 1990, the thoracic viscera of culled buffalo were no longer presented for meat inspection at the abattoir. This followed a request from the National Parks Board to allow the removal of the thoracic organs as well as the abdominal viscera in the field, in order to solve the significant problem of post mortem decomposition in transported carcasses. This request was approved in the light of the fact that no significant lesions, from a public-health point of view, had been found in the thoracic organs of buffalo during the preceding 10 years.

The source of this disease outbreak and its manner of entrance into the KNP, remain speculative. From annual reports of the State Veterinarian Barberton/Nelspruit (Anon. 1963, 1983, 1984) it could be determined that significant outbreaks of bovine tuberculosis had occurred in cattle on farms along the Crocodile River, which forms the southern boundary of the KNP, during the early 1960s and again in 1982, 1983 and 1984. During both periods, outbreaks of Corridor disease (buffalo-associated theileriosis) occurred in cattle in this same area, indicating that there had been close contact between cattle and buffalo. Transmission of bovine tuberculosis from cattle to buffalo could have taken place during one of these two periods. If the disease had entered the KNP during the earlier outbreak (1960s) it could have remained at undetectable levels for years until the prevalence rates in herds had increased significantly.

Recently (1992/1993), intensive tuberculin skin testing of tribal cattle bordering the western and south-western corner of the KNP, failed to detect any

TABLE 1 Cultural and biochemical characteristics of acid-fast bacteria isolated from buffalo

Growth rate at 27°C 37°C 45°C	No growth Slow No growth
Stimulation by pyruvate Pigmentation Niacin production Nitrate reduction Catalase production Tellurite reduction Thiophene carboxylic-acidhydrazide	Positive Negative Negative Negative Negative Negative Sensitive

tuberculosis in these herds, making them an unlikely source of infection (P. Smith, personal communication 1993).

A paper reporting full details of the epidemiological features of the disease, following a more comprehensive follow-up sampling of most buffalo herds throughout the entire KNP, is currently in preparation.

REFERENCES

- ANON. 1963, 1983, 1984. *Annual Reports, State Veterinarian: Barberton/Nelspruit*.
- ANON. 1983. *Laboratory manual: Tuberculosis Laboratory, Bacteriology*. Onderstepoort Veterinary Institute.
- BASSON, P.A., McCULLY, R.M., KRUGER, S.P., VAN NIEKERK, J.W., YOUNG, E. & DE VOS, V. 1970. Parasitic and other diseases of African buffalo in the Kruger National Park. *Onderstepoort Journal of Veterinary Research*, 37:11–28.
- CLANCEY, J.K. 1977. The incidence of tuberculosis in lechwe (Marsh Antelope). *Tubercle*, 58:151–156.
- DE VOS, V., McCULLY, R.M. & VAN NIEKERK, C.A.W.J. 1977. Mycobacteriosis in the Kruger National Park. *Koedoe*, 20:1–9.
- DE VOS, V., BENGIS, R.G. & COETZEE, H.J. 1983. Population control of large mammals in the Kruger National Park, in *Management of large mammals in African conservation areas*, edited by P.N. Owen-Smith. Pretoria: HAUM Educational Publishers: 213–231.
- GALLAGHER, J., MACADAM, I., SAYER, J. & VAN LAVIEREN, L.P. 1972. Pulmonary tuberculosis in free living lechwe antelope in Zambia. *Tropical Animal Health and Production*, 4:204–213.
- GUILBRIDE, P.D.L., ROLLISON, D.H.L., McANULTY, E.G., ALLEY, J.G. & WELLS, J.G. 1963. Tuberculosis in the free living African (Cape) buffalo (*Syncerus caffer*, Sparrman). *Journal of Comparative Pathology and Therapeutics*, 73:337–348.
- HOFMEYR, C.F.B. 1956. Two hundred and eighty-four autopsies at the National Zoological Gardens, Pretoria. *Journal of the South African Veterinary Medical Association*, 27:263–266.
- KEEP, M.E. & BASSON, P.A. 1973. Mycobacteriosis in black rhinoceros (*Diceros bicornis* Linnaeus, 1758). *Journal of the South African Veterinary Association*, 44:285–286.
- MARTINAGLIA, G. 1930. A strain of *Mycobacterium tuberculosis* from the giraffe and further observations of *M. tuberculosis* from the koodoo and duiker. *16th Report of the Division of Veterinary Services and Animal Industry*: 143.
- MONTALI, R.J. 1978. Ed. Mycobacterial infections of zoo animals. *Proceedings of a symposium on mycobacterial infections of zoo animals held at the National Zoological Park, Smithsonian Institution*.
- NEL, E.E., KLEEBERG, H.H. & GATNER, E.M.S. 1980. *Laboratory manual of tuberculosis methods*. 2nd revised ed. Tuberculosis Research Institute. SAMRC. Pretoria.
- PAINE, R. & MARTINAGLIA, G. 1928. Tuberculosis in wild buck living under natural conditions. *Journal of the South African Veterinary Medical Association*, 1:87.
- ROBINSON, E.M. 1944. A note on strains of tuberculosis from the Cape kudu. *Onderstepoort Journal of Veterinary Science and Animal Industry*, 19:23.
- ROBINSON, E.M. 1953. A few cases of tuberculosis. *Journal of the South African Veterinary Medical Association*, 24:97.
- THORNBURN, J.A. & THOMAS, A.D. 1940. Tuberculosis in the Cape kudu. *Journal of the South African Veterinary Medical Association*, 11:3.
- THURLBECK, W.M., BUTAS, C.A., MANKIEWIC, E.M. & LAWS, R.M. 1965. Chronic pulmonary disease in the wild buffalo (*Syncerus caffer*) in Uganda, in *American Review of Respiratory Diseases*, 92:801–805.
- WOODFORD, M.H. 1982(a). Tuberculosis in wildlife in the Ruwenzori National Park, Uganda. I. *Tropical Animal Health and Production*, 14:81–88.
- WOODFORD, M.H. 1982(b). Tuberculosis in wildlife in the Ruwenzori National Park, Uganda. II. *Tropical Animal Health and Production*, 14:155–160.