

MYCOBACTERIOSIS IN THE KRUGER NATIONAL PARK*

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Abstract – A fatal case of mycobacteriosis in a free-ranging impala *Aepyceros melampus* from the Kruger National Park (KNP) is described. A description of the macro- and microscopical manifestations is given. This is the first report of mycobacteriosis in the KNP and in the impala. In a subsequent survey on 27 939 animals, which represents five different big game species from the KNP, negative results for mycobacteriosis were obtained. In the light of these findings it is conjectured that the one positive case in the impala was incidental with the source of infection unknown. The possibility of it being due to the avian tubercle bacillus is, however, raised.

Introduction

The host range of mycobacteriosis in wild animals appears to be unlimited (Francis 1958). It has also become recognized as a common disease of wild animals, particularly those closely associated with man and his environment, such as zoological gardens (Winkler and Gale 1970). A few such cases have been diagnosed in the Republic of South Africa, viz.

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springbok *Antidorcas marsupialis* (Robinson 1953; Hofmeyr 1956), giraffe *Giraffa camelopardalis* (Martinaglia 1930; Basson, McCully, Kruger, van Nickerk, Young, De Vos, Keep and Ebedes 1971b), black rhinoceros *Diceros bicornis*, buffalo *Syncerus caffer* and nyala *Tragelaphus angasi* (Hofmeyr 1956). In all of these cases death was attributed to *Mycobacterium bovis*, with typical symptoms and lesions noted.

The first report of an outbreak of mycobacteriosis in free-living game animals in South Africa was given by Paine and Martinaglia in 1928. They described the disease in kudu *Tragelaphus strepsiceros* and a duiker *Sylvicapra grimmia*, the causal organism being *Mycobacterium tuberculosis* var. *bovis*. Evidence of deaths with abscess formation among springbok, duiker, bushbuck *Tragelaphus scriptus* and hares *Lepus* spp. in the vicinity of the outbreak, were also presented by the authors.

A condition in the kudu that was known for many years in the farming community of the "Grahamstown bush veld", was reported as tuberculosis in 1940 by Thorburn and Thomas (1940). Robinson (1944) later showed that the strain of *Mycobacterium tuberculosis* involved in this outbreak, was bovine in type.

Another case of tuberculosis in the wild is described by Guilbride, Rollinson, McAnulty, Alley and Wells (1963) when they found eight cases of tuberculosis among 13 free-living buffalo shot in the Queen Elizabeth National Park in Uganda. The authors maintained that tuberculosis in cattle bordering on the Park was extremely high and although it was not possible to ascertain the direct connection between such cattle and buffalo, it was presumed that occasions arise where cattle herds are grazed in the same area as buffalo.

Recently a case of mycobacteriosis in a free-ranging black rhinoceros from the Hluhluwe Game Reserve has been reported by Keep and Basson (1973).

No previous indication as to the occurrence of the disease in wild animals from the Kruger National Park (KNP) could be found in the literature or departmental reports. The impala has also not yet been named as a victim of the disease. When a case of mycobacteriosis was therefore found in an impala from the KNP, it triggered off a close inspection routine of all carcasses which subsequently became available. This report documents the findings of the positive case in the impala and the results of subsequent inspection, or the incidence of the disease in the Park.

Material and Methods

An impala carcass which was found in the veld was subjected to full necropsy procedures and material from lesions collected in 10% formalin for later histological examination. The formalin-fixed tissues were prepared in a routine manner for light microscopy. The haematoxylin and eosin (HE) and Ziehl-Neelsen (ZN) methods of staining were used (Cruickshank 1962).

All carcasses which became available as a result of the game control

programme in the KNP were subjected to the standard routine slaughter house inspections for mycobacteriosis. Suspect lesions were collected and smears, stained the Ziehl-Neelsen method (Cruickshank 1962) were examined under ordinary light microscopy.

Results

History

While doing a routine inspection drive of the Crocodile Bridge Ranger section of the KNP during November 1967 a carcass of an impala was found on the brink of an earthen dam about 10 km north of the Crocodile River, the southern border of the Park. A necropsy was performed on the spot.

Macroscopic Examination

The carcass was that of an adult male impala. It was still warm to the touch, indicating that death must have taken place shortly before arrival. Very few signs of pre-death agonal movements were noticed and the carcass was still completely intact with no signs of interference by scavengers. On external examination the general impression was gained that the animal was in a very good condition. On closer inspection a prolific swelling of the superficial lymph nodes especially the prescapular and mandibular, was apparent. No signs of fistula formation could be seen.

On opening up the carcass it was clear that virtually all organs were affected by firm white granulomatous nodules varying in size from less than one mm to several centimetres in diameter as can be seen in Fig. 1. On sectioning the nodules they gave the impression of proliferative granulomatous material with a white fatty appearance. Some of them exhibited small caseous centres. No gritty sensation or grating sound was discernible while cutting into the lesions. The lungs were invaded by countless miliary nodules, some of them coalescing to form bigger lesions, such as demonstrated by Fig. 1. Lung lesions were, however, complicated by inflammatory changes due to a heavy infestation of lung-worms *Pneumostrongylus calcaratus*.

On gross examination the liver, spleen, kidney and adrenals appeared to be less severely affected. Nodules were nevertheless distributed throughout the tissue, some of them forming a distinct bulge on the surface.

Disseminated nodules over the pleural and peritoneal surfaces were also visible, the appearance resembling the so-called "pearl disease" manifestation of tuberculosis in cattle as described by Smith, Jones and Hunt (1972).

The lymph glands were enlarged and on cut section appeared oedematous and proliferative, the most severely affected being the mandibular, prescapular, bronchial, portal and mesenteric glands. In addition the mandibular and bronchial glands showed signs of the same type of lesion as found in the various organs.

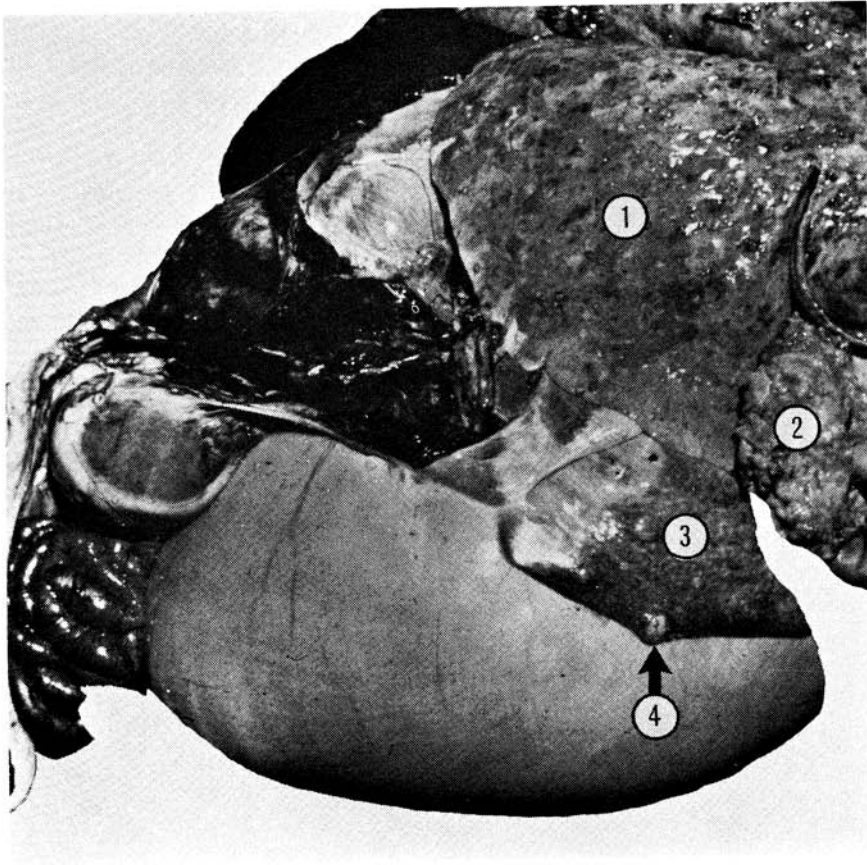


Fig. 1. Some gross features of mycobacteriosis in an impala from the Kruger National Park. 1. Lung with miliary distribution of nodules due to mycobacteriosis; some coalescing and combining with lesions due to *Pneumostrongylus calcaratus* lungworm to form large organised areas (2). 3. Spleen with tubercular nodules, some bulging out above the surface (4).

Microscopic Examination

Lungs, spleen, lymph nodes, omentum/mesentery, liver, brain and meninges were examined histologically with only the latter two free of lesions. The lungs contained lesions due to an acid-fast bacterium and to the nematode *Pneumostrongylus calcaratus*. In some areas there was a mixture of the adults, ova and larvae of the nematode and numerous acid-fast bacilli. A granulomatous response and large areas of caeseation necrosis were present. Within the caeseated areas there were many nematode eggs and larvae. Near the sharp border of one lung there was a very large area of caeseation necrosis which suggested that vascular obstruction may have contributed to its pathogenesis. In all of these areas

there were numerous acid-fast bacilli, and in some giant cells both larvae of the nematode and acid-fast bacilli were present. There were other areas of caeseation necrosis and granulomatous reaction where no stage of the nematode was observed. These areas varied from miliary foci of epithelioid cells, typical miliary tubercles, to large granulomata with caeseous centres partially mineralized. The reaction around the caeseous centres was composed of epithelioid and giant cells. The cytoplasm of some groups of epithelioid cells was confluent presenting the appearance of huge giant cells. In the miliary tubercles acid-fast organisms were rare but in the large granulomata they were numerous.

The spleen was very severely affected. There were many large areas of granulomatous inflammation characterized by centres of caeseation necrosis with mineralization. Surrounding the caeseous centres there was a diffuse proliferation of fibroblasts, fibrocytes and epithelioid cells with an intense component of Langhans' giant cells. The large granulomas were separated from the normal architecture of the spleen by a capsule of fibrovascular tissue composed of mature and immature connective tissue and an admixture of epithelioid and giant cells. Breaking through the capsule at intervals, the infection was causing smaller satellite tubercles. At other sites in the spleen there were more bland early, granulomatous reactions at the location of haematogenously distributed acid-fast organisms.

With the ZN stain acid-fast bacteria were very numerous in the large granulomas and far less numerous in the other lesions.

The two enlarged lymph nodes examined, consisted essentially of a dense fibrous capsule around a large area of caeseation necrosis that occupied almost all the space with the exception of a small area of cortex in one and medulla of the other lymph node. Mineralization was present in the caeseated area. On the edge of the caeseated necrotic lesion there was a massive proliferation of epithelioid and giant cells that gradually blended in with the architecture of the remaining lymph node. The peripheral and medullary sinuses, where recognizable, were filled with epithelioid and giant cells. ZN stains revealed many acid-fast bacilli but they were not as numerous as in the spleen.

In the mesentery/omentum there were numerous large granulomas with centres of caeseation necrosis and mineralization. Epithelioid and giant cells were the predominant cells immediately surrounding the caeseation necrosis. At the periphery of the granulomas, there was an intense fibrovascular response. This indicated extensive involvement of the mesentery and omentum. Acid-fast organisms were less numerous than in the spleen, lymph node and lung.

Of the tissue examined that contained lesions, the liver was the least involved, but in each liver section there was at least one large granuloma that involved one or two lobules. These had mineralized centres of caeseation necrosis that were surrounded by a zone of epithelioid and giant cells. Beyond that zone was a fibrovascular capsule permeated by lymphocytes. The outermost part of the capsule was dense collagen. There

were numerous acid-fast organisms in the epithelioid and giant cells.

Elsewhere there were a few miliary granulomas widely disseminated in the liver parenchyma. They were composed of mainly epithelioid cells and a few giant cells. Acid-fast organisms were very scarce in these microgranulomas.

The acid-fast organisms varied considerably in size from very short rods to longer organisms equivalent to about four impala erythrocytes in the sections examined. Some were beaded in appearance and slightly comma shaped. There were no filaments nor branching organisms.

Considerable search failed to reveal the presence of acid-fast organisms within either ova or larvae of the lungworm. Intestines of adults, however, invariably contained fragments of acid-fast material in a size range which could be fragmented organisms.

From 1967.04.01 to 1974.03.31 a total of 11 985 impala, 2 067 blue wildebeest *Connochaetes taurinus*, 8 701 buffalo, 5 031 elephants *Loxodonta africana* and 155 hippopotami *Hippopotamus amphibius* were inspected for mycobacteriosis; a total of 27 939 animals. Of this total 2 323 impala were obtained from the close vicinity of the positive mycobacteriosis case in the impala. Negative findings were obtained throughout. A few suspect lesions were found but gave negative results on closer examination.

Discussion

The macro- and microscopic characteristics coupled with the occurrence of a small acid fast organism in the lesions warrants a diagnosis of generalized mycobacteriosis, but of undetermined type. Judging from the disseminated proliferative granulomatous nature of the disease and limited mineralization, the disease must have run a fairly subchronic course. The good condition of the animal further indicates a fairly good resistance. The cause of death can nevertheless be ascribed to mycobacteriosis.

With the exception of the black rhino (Keep and Basson 1973) the previous cases of mycobacteriosis in the wild were associated with communal grazing and watering systems between wild and domestic animals (Paine and Martinaglia 1928; Smith *et al* 1972; Guilbride *et al* 1963). In this case such an association was impossible, the nearest border of the KNP and concomitant domestic animal activity being 10 km in a straight line from the spot where the carcass was found. A game fence and river along the border further lessened the chances of contact. It must therefore be accepted that the animal contracted the disease from a reservoir in nature. This realization actually instigated a closer inspection of wild animal carcasses and material which were destined for the slaughter house and human consumption. Consistent negative findings from substantial numbers of impala, blue wildebeest, buffalo and elephant, however, indicate a negligibly low incidence of the disease for these species in the Park. This is further corroborated by intensive species pathology surveys which were performed on hippopotamus

(McCully, Van Niekerk and Kruger 1967), buffalo (Basson, McCully, Kruger, Van Niekerk, Young and De Vos 1970), elephant (Basson, McCully, De Vos, Young and Kruger 1971a) and chacma baboon *Papio ursinus* (McConnell, Basson, De Vos, Myers and Kuntz 1974; De Vos, Van Niekerk and McConnell 1973) populations in the Park. It must therefore be accepted that the lone positive case in the impala was due to an incidental infection or was an aberrant case with the reservoir still an unknown entity.

Similar low incidence results in free-living mammals were obtained by Sachs (1968) who commented on the absence of mycobacteriosis in African game animals from the Serengeti National Park area of Tanzania. These findings further lends support to the theory advanced by Henning (1956): "As long as wild animals which are susceptible to the disease, are kept under natural conditions in the open country infection seldom occurs; but when they are detained in captivity they are liable to become infected".

The large number of acid-fast organisms in this impala, especially in the spleen, lung, and lymph nodes, suggests that this may represent infection by the avian tubercle bacillus. For that reason a case of avian tuberculosis in a bovine known to have been caused specifically by the avian tubercle bacillus, based on specific cultural identification, was used for comparison through the courtesy of G. Imes, AFIP, Washington, D.C. There were many similarities between this bovine case and the impala. The host reactions were essentially the same with the epithelioid cell present in almost "pure culture" in some areas of affected tissues of both animals. There were, however, far more acid-fast organisms in the bovine than in the impala.

The extent to which avian tuberculosis occurs in bird life in the KNP, especially in migratory species, is not specifically known. That an affected bird could have infected this impala by excrement thus cannot be excluded and in view of the scarcity of other possible sources of tuberculosis, it remains an attractive possibility.

It also bears to note that tissue reaction due to severe lungworm infestation might complicate and even camouflage the diagnosis of lung mycobacteriosis in the impala. In a survey on mycobacteriosis this distinct possibility should be kept in mind.

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