Tuberculosis in Kafue lechwe (*Kobus leche kafuensis*) and in a bushbuck (*Tragelaphus scriptus*) on a game ranch in Central Province, Zambia

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ABSTRACT

Mycobacteriosis was diagnosed for the first time outside a national park in free-ranging wild animals on a game ranch in Zambia. A Kafue lechwe (*Kobus leche kafuensis*) was found dead with tuberculous lesions on a ranch near Lusaka. Acid-fast bacilli were found in the affected organs. Mycobacteria were isolated from these tissues. A bushbuck (*Tragelaphus scriptus*) was found dead on the same ranch with multiple superficial abscesses in the neck region, extensive granulomatous lesions in the lung, the bronchial and mediastinal lymph nodes and several nodular lesions in the spleen. Few acid-fast bacilli were found in the exudate from the abscesses and lesions in the affected organs. Histologically the lesions resembled those of tuberculosis, but mycobacteria could not be isolated. In addition, 1 Kafue lechwe among 37 wild ungulates of 13 species shot on the ranch showed typical tuberculous lesions in the lungs, but the diagnosis was not confirmed by bacterial isolation. The role of the Kafue lechwe as maintenance host for tuberculosis as well as in the possible spread of this disease to other wildlife are discussed.

Key words: bushbuck (*Tragelaphus scriptus*), Kafue lechwe (*Kobus leche kafuensis*), mycobacteriosis, tuberculosis, wildlife, Zambia.

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INTRODUCTION

Tuberculosis, caused by *Mycobacterium bovis,* has been described in a variety of captive and free-ranging wild animals. Among the latter, self-maintaining infections have been reported in the European badger (*Meles meles*) in the United Kingdom; in the brush-tailed possum (*Trichosurus vulpecula*) in New Zealand and in farmed fallow deer (*Dama dama*) as well as red deer (*Cervus elaphus*) in several other countries[3,4,6,23,25]. The first cases of bovine tuberculosis in free-ranging wild animals in South Africa were reported in 1928 in greater kudu (*Tragelaphus strepsiceros*) and common duiker (*Sylvicapra grimmia*)[18]. Greater kudu were later found to be endemically infected with *M. bovis* in the Eastern Cape region of South Africa[24]. Tuberculosis caused by *M. bovis* is endemic in African buffalo (*Syncerus caffer*) and in warthog (*Phacochoerus aethiopicus*) populations in the Queen Elizabeth National Park in Uganda[9,26,27]. High prevalence rates of *M. bovis* infections were found in wild olive baboons (*Papio cynocephalus anubis*) in the Masai Mara Game Reserve in Kenya[20]. Recently, an outbreak of tuberculosis in a free-ranging African buffalo population and subsequent spread of this disease to other species was reported from the Kruger National Park, South Africa[2,12,13].

In Zambia, tuberculosis caused by *M. bovis* in free-ranging wild animals has been reported only in a single eland (*Taurotragus oryx*)[15] and in Kafue lechwe (*Kobus leche kafuensis*)[5,8,15]. It was suggested that tuberculous cattle that shared the grazing on the Kafue flood plains with lechwe introduced this disease to the susceptible wildlife population[8].

In 1989 the legislation in Zambia pertaining to wildlife was amended and many private game ranches have since been established. Several ranches were stocked, amongst other species, with Kafue lechwe that had been captured on the Kafue flood plains. This report presents 3 cases of tuberculosis or mycobacteriosis, 2 in Kafue lechwe and 1 in a bushbuck from a private game ranch near Lusaka. The pathological and histological lesions were typical of tuberculosis in all 3 cases; however, mycobacteria could only be isolated from 1 of the Kafue lechwe. Unfortunately, appropriate biochemical tests were not available at the time to confirm without doubt that the isolate was *M bovis*. The strong suspicion, however, that bovine tuberculosis is present in wildlife on private Zambian ranches warrants attention. The danger of spreading tuberculosis through wildlife translocations is emphasised.

MATERIALS AND METHODS

Animals

All animals came from a private game ranch (15.05 S, 28.15 E) located in the Chisamba District in the Central Province of Zambia, approximately 20 km north of Lusaka. This ranch has operated commercially as a game ranch since 1990, when wildlife was first introduced. Eighteen ungulate species are present at a stocking rate of approximately 1 large stock unit per 4.7 ha[16].

A male Kafue lechwe was found freshly dead on the ranch in November 1996. The carcass was transported immediately to the Disease Control Laboratory of the School of Veterinary Science, University of Zambia, for a *post mortem* examination. This animal had been dead for presumably 5–6 hours when the necropsy was conducted.

A male bushbuck (*Tragelaphus scriptus*) was found dead on the same ranch in September 1996. A necropsy was performed immediately on site. It was estimated that the bushbuck had been dead for about 2–3 hours when the necropsy was conducted. All organs that contained lesions were cooled at 4 °C and transported to the same laboratory for diagnosis.

In addition, 37 wild ungulates were shot on the ranch between December 1995 and November 1996 for venison, as trophies or because of injuries. These animals included 1 Burchell's zebra

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(*Equus burchellii*), 2 bushpigs (*Potamochoerus larvatus*), 11 impala (*Aepyceros melampus),* 3 tsessebe (*Damaliscus lunatus lunatus*), 1 Lichtenstein's hartebeest (*Sigmoceros lichtensteinii*), 2 eland, 2 bushbuck, 4 greater kudu, 2 sable antelope (*Hippotragus niger*), 6 defassa waterbuck (*Kobus ellipsiprymnus defassa*), 1 Kafue lechwe, 1 puku (*Kobus vardonii*) and 1 reedbuck (*Redunca arundinum*). All carcasses were transported immediately after death to a nearby abattoir. In addition to standard meat inspection procedures, the thoracic and visceral organs were examined more thoroughly according to standard necropsy procedures.

Gross and histopathology

All observed abnormalities were recorded while the necropsies were performed. Specimens of selected organs were preserved in 10 % formol saline. These were later routinely processed and stained with haematoxylin and eosin for histological examination. Selected tissue sections as well as smears of exudates were stained with the Ziehl-Neelsen stain for the detection of acid-fast bacteria.

Bacteriology

Specimens of affected organs were collected and processed for bacterial isolation and identification[1]. Petroff's modified concentration method was used for the preparation of the inoculum. The inoculum was smeared over the surface of each tube of Lowenstein-Jensen (LJ) egg medium containing glycerine, LJ egg medium without glycerine and LJ egg medium containing pyruvate. These tubes were incubated aerobically at 37 °C and checked for growth of colonies of acid-fast bacteria after 2 weeks and then every week for 8 weeks. The isolates were further identified by testing their niacin production and by their sensitivity to thiophene carboxylic acid hydrazide.

RESULTS

Natural deaths

Kafue lechwe

The carcass was severely emaciated. All lobes of the right lung and the diaphragmatic lobe of the left lung were consolidated. The mediastinal lymph nodes were slightly enlarged but lacked gross pathological changes. There was mild enteritis and the mesenteric lymph nodes were slightly enlarged. Both kidneys revealed large, encapsulated, white, partially calcified abscesses varying in diameter from 20 to 60 mm with central necrosis. Approximately 70 % of the normal kidney tissue was replaced by calcified, white necrotic material that was hard and gritty to cut. The testes revealed a few yellowish nodules on their surface. The urinary bladder was full of turbid urine. The visceral surface of the bladder showed a few whitish, pinhead-sized nodules.

Large numbers of acid-fast bacilli were seen in the smears made from the kidneys and the urine sediment while few acid-fast bacteria were present in the impression smears made from the lungs and the mediastinal lymph nodes. Microscopically the lesions were characterised by large aggregates of epithelioid cells and the lack of Langhan's giant cells. Few acid-fast bacilli were detected in the cytoplasm of epithelioid cells. The lesions were typical of tuberculosis. With the exception of severe testicular atrophy, no other lesions were seen in the testis.

Colonies of acid-fast bacteria were seen after 5 weeks of incubation on the LJ media slants containing pyruvate inoculated with material extracted from the kidneys, urine and lungs. The colonies were white and had a smooth surface. No growth was observed on media either with or without glycerine during 8 weeks of incubation. The culture was found to be niacin-negative and sensitive to thiophene carboxylic acid hydrazide.

The findings that these colonies appeared only after 5 weeks of incubation, were not pigmented and smooth, grew only on pyruvate-enriched but not on glycerine-containing media, and were TCH sensitive indicate that the isolate was most probably *M. bovis.* However, a final differentiation from other members of the *M. tuberculosis* complex (*M. tuberculosis, M. africanum* and *M. microti*) was not achieved as other biochemical tests (pyraziamide susceptibility, nitrate reduction, catalase production, tellurite reduction) were not available at the time.

Bushbuck

The carcass was severely emaciated. Several large, superficial abscesses were visible in the submandibular and retropharyngeal regions, along the neck and in the prescapular region. The abscesses varied from approximately 60 to 150 mm in diameter. Two abscesses discharged a yellowish-white exudate through cutaneous sinuses. On cut surface, all abscesses contained a similar exudate. Both lungs contained multiple firm nodules varying from 5 to 70 mm in diameter. On cut surface, numerous necrotic foci were evident in the nodules. The thoracic lymph nodes were grossly enlarged and up to 120 mm in diameter. Most of them were firm and contained necrotic material while 1 contained a yellowish-white exudate. The spleen showed disseminated miliary lesions, many of which were calcified.

The impression smears made from exudate obtained from the abscesses revealed only few acid-fast bacteria. Microscopically the lesions in the lungs, lymph nodes and the spleen were similar in appearance. The lesions consisted of large masses of caseous necrotic material enveloped by an attenuated granulomatous inflammatory reaction. Scattered foci of calcification occurred in the necrotic tissue. The lesions were further characterised by the presence of numerous neutrophils in the reaction. Few scattered, acid-fast bacilli were seen in the cytoplasm of Langhan's giant cells and in the necrotic debris. These findings suggest a diagnosis of tuberculosis.

No growth was obtained on LJ medium during 10 weeks of incubation.

Culled animals

Typical tuberculous lesions were found in 1 of the 37 animals that were shot. This was a 3-year-old Kafue lechwe, which was culled because of a hind-leg injury but appeared otherwise healthy. Several firm nodules varying between 30 and 55 mm in diameter were found in the lung tissue. On cut surface, these nodules had caseous necrotic centres. Histologically these lesions reflected multifocal, necrogranulomatous pneumonia characterised by large central calcified cores of cellular debris surrounded by attenuated collars of granulomatous inflammatory reaction. The lesions were well-encapsulated and surrounded by areas of atelectasis. Histologically the lesions resembled those of tuberculosis. However, the diagnosis could not be confirmed as no acid-fast bacteria were detected on Ziehl-Neelsen-stained sections.

DISCUSSION

Bovine tuberculosis has previously been reported from free-living Kafue lechwe in Zambia[5,8,15]. This is the 1st case of tuberculosis suspected to be caused by *M. bovis* infection in a Kafue lechwe from a private game ranch in Zambia. Mycobacteriosis was also diagnosed in a free-ranging bushbuck for the 1st time. Although the microbiological isolation failed in this case, the typical pathological and histological lesions and the presence of acid-fast bacilli in these lesions are consistent with those of tuberculosis.

The occurrence of tuberculosis in the Kafue lechwe does not come as a surprise.

Kafue lechwe are endemic to the Kafue flats in Zambia and it has been well known since 1972 that prevalence rates of *M. bovis* infections in this population are high. Prevalence rates of 36.0 %, 33.0 % and 43.4 %, respectively, were found in 3 extensive surveys that were conducted here[5,8,15]. Free-living lechwe have been captured on the Kafue flats and translocated to private game ranches within Zambia since late 1989 without having been tested for tuberculosis.

It is likely that lechwe now serve as constant sources of infection for other animals on private ranches. The reported high prevalence rates in Kafue lechwe indicate that the infection can be self-maintaining in these populations. *M. bovis* has an exceptionally wide host range and the presence of 1 maintenance host in an ecosystem is sufficient to spread bovine tuberculosis to other susceptible hosts[10,17]. This has been shown in the United Kingdom, where tuberculous badgers serve as the disease reservoir for cattle[4] and in New Zealand where tuberculous possums persistently re-infect cattle[6]. Recently, free-ranging buffalo in the Kruger National Park, South Africa, have been incriminated as the source of bovine tuberculosis, spreading the disease to other hosts such as lion (*Panthera leo*), cheetah (*Acinonyx jubatus*) and chacma baboon (*Papio ursinus*)[13]. The bushbuck reported here must have contracted tuberculosis on the game ranch. This animal was only 3 years old and no animals had been introduced after 1991. It is likely that the bushbuck contracted the disease from the lechwe population.

The mode of transmission of *M. bovis* among wildlife remains speculative. Possible routes of infection are respiratory, alimentary, congenital, cutaneous and venereal[11]. In cattle, infection is acquired almost exclusively aerogenously by the inhalation of infected droplets from an animal with open pulmonary tuberculosis or from infected dust particles. Usually close contact between infected and non-infected animals is required[7]. However, tuberculosis has been found in extensively farmed cattle in southern Africa and Australia, possibly owing to their habit of congregating in large numbers at watering points under dusty conditions[11]. Kafue lechwe are gregarious ungulates that utilise a semi-aquatic habitat throughout the year[21]. On the game ranch under investigation they occupy a small area around 1 of the few permanent water sources. Large numbers of wildlife congregate here, particularly during the dry season. The close contact between the Kafue lechwe and other susceptible wild animals during this time may have facilitated the spread of the disease *via* droplet infection and the bushbuck could thus have been infected. Tubercle bacilli can also be shed *via* urine, faeces, vaginal secretions, semen or discharging abscesses and thus contaminate soil, water and plants[14,19]. The survival time and infectivity of these organisms in the environment have recently been reviewed in detail by Morris *et al.*[17]. The practical significance of persistence of tubercle bacilli in the environment as a source of infection appears to be small. However, the bacilli can remain infective for several days depending on climatic conditions and this may contribute to the spread of the disease through oral and/or percutaneous infection of new hosts. Percutaneous infection as a result of scratching the ears with contaminated hind legs has been described in kudus in the Eastern Cape Province in South Africa[24].

Tuberculosis in bushbuck is most probably not self-maintaining as these animals usually live a solitary life style for most of the year[22]. Spread of the disease will consequently be slow and the bushbuck can thus be considered a spill-over host. However, with 1 maintenance host being present, it is possible that tuberculosis could manifest itself also in gregarious animals other than Kafue lechwe. It appears that this has not yet occurred on the game ranch investigated, as none of the other animals examined showed tuberculous lesions.

Further tests are still required to confirm the presence of *M. bovis.* The suspicion that bovine tuberculosis is present in wildlife on private Zambian game ranches should be followed up, as tuberculosis is considered a serious health hazard. Some of the wild ungulates kept on Zambian ranches, such as Lichtenstein's hartebeest, roan antelope (*Hippotragus equinus*) and sable antelope, are sought after as breeding animals for other ranches within the country and for export. Veterinary control measures must be considered and implemented urgently before bovine tuberculosis becomes widely established on game ranches and poses a threat to the country's wildlife industry.

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