Case report — Gevalverslag

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 is discussed.

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


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\textsuperscript{1-3,5,12,15}. 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)\textsuperscript{5}. Greater kudu were later found to be endemically infected with M. bovis in the Eastern Cape region of South Africa\textsuperscript{4}. 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\textsuperscript{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\textsuperscript{30}. 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\textsuperscript{25,31,32}.

In Zambia, tuberculosis caused by M. bovis in free-ranging wild animals has been reported only in a single eland (Taurotragus oryx)\textsuperscript{16} and in Kafue lechwe (Kobus leche kafuensis)\textsuperscript{17,18}. 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\textsuperscript{7}.

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\textsuperscript{1}\textsuperscript{2}.

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.
(Equus burchelli), 2 bushpigs (Potamochoerus larvatus), 11 impala (Aepyceros melampus), 3 tsessebe (Damalesic lupinus lunatus), 1 Lichtenstein’s hartebeest (Sig- nucoerus lichtensteinii), 2 eland, 2 bushbuck, 4 greater kudu, 2 sable antelope (Hippotragus niger), 6 defassa waterbuck (Kobus ellipsoptyrmmis defassa), 1 Kafue lechwe, 1 puku (Kobus vardoni) 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 % formal 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. 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 consoli- dated. 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 vesical 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 tests.

Colonies of acid-fast bacteria were seen after 5 weeks of incubation on the LJ medium slants containing pyruvate inocu- lated 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 (pyrazamide 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 granulomato- tous 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 cyto- plasm 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-encapsu- lated and surrounded by areas of atelecta- sis. 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. This is the 1st case of tubercu- losis 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 histo- logical 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. 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 is likely that this has not yet occurred on game ranches.

The mode of transmission of M. bovis among wildlife remains speculative. Possible routes of infection are respiratory, alimentary, congenital, cutaneous and venereal. 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. 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. Kafue lechwe are gregarious ungulates that utilise a semi-aquatic habitat throughout the year. 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. The survival time and infectivity of these organisms in the environment have recently been reviewed in detail by Morris et al. 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.

Tuberculosis in bushbuck is most probably not self-maintaining as these animals usually live a solitary life style for most of the year. 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 Kafue lechwe antelope (Hippotragus niger) and chama baboon (Papio ursinus). 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 before 1991. It is likely that the bushbuck contracted the disease from the lechwe population.

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Book review — Boekresensie

Genetic resistance to animal diseases

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This special issue of the Scientific and Technical Review of the OIE (Office International des Épizooties – the world organisation for animal health) provides a compendium of 27 papers on a subject of growing importance for the worldwide future of livestock health and production, more especially in Africa. Its major appeal will lie primarily in the rare quality of many of the papers and the balance it strikes in presenting the scientific basis of disease resistance and the methods – from conventional breeding to gene transfer – that can be employed to achieve it. A minor deficiency is the lack, as far as one can judge, of a single African among the 59 contributors and the passing lip-service it pays to mammalian diversity in Africa. That diversity contains much of the genetic potential for addressing a whole range of infectious disease problems in Africa and elsewhere.

Genetic resistance to animal disease, i.e. the heritable diversity in the susceptibility of species, breeds and individuals, has been recognised for a long time, but has only been fitfully exploited. Perhaps the best local examples were the pioneering observations of J.C. Bonma on differences between breeds of cattle to tick infestation and heartwater and the subsequent development of the Bonsmara breed. Genetic variability in the resistance of poultry breeds to Marek’s disease and avian leukosis has also long been appreciated. A more recent example from Australia, described in this volume, is aimed at the problems of gastrointestinal nematode infestations, flystrike (cutaneous myiasis) and footrot in Merino sheep (Raadsma, Gray and Woolaston). Other than these, however, practical success stories, as opposed to interesting scientific phenomena, are hard to find.

Therein lies this volume’s probable value, i.e. in successfully making the major issues with respect to disease resistance available to a wide audience concerned with the control of animal disease. It adequately demonstrates the paucity of progress made because of the complexity of the subject but, at the same time, points out the future potential. The realisation of this potential is arguably more important for Africa than it is for other regions of the world because financial, logistical and political realities are making the conventional control of animal diseases in Africa increasingly difficult and sometimes impossible to implement. Genetic resistance to disease may help to ameliorate these constraints in some circumstances.

From a personal perspective the chapters on the biological principles of heredity and resistance to disease (Horin) and genomic approaches to the improvement of disease resistance in farm animals (Soller and Andersson) were particularly useful. It could be argued from the same perspective that there are too many papers dealing with conventional immunological topics such as non-specific immunity, immunoglobulin diversity, T-cell receptors, cytokines and major histocompatibility complexes because this information is available from other sources. On the other hand, there has been a clear effort in the selection of these papers to concentrate on farm animals rather than using mouse and human models or examples that one finds regularly in texts purporting to address animal diseases. These chapters are therefore likely to be a ready source of summarised information for non-specialists.

The papers are divided into 9 groups, viz. mechanisms of defence against infectious diseases; genetic resistance to parasites; bacteria; viruses; prions; conventional breeding programmes; marker-assisted selection and identification of disease traits; study of genetic resistance by targeted disruption of gene function; and genetic resistance through gene transfer. Some groups, however, contain only one paper. The papers have obviously been carefully edited because few editorial errors were encountered and the papers themselves are clear and easy to read.

For anyone interested in disease resistance in farm animals this volume contains a fund of useful and interesting information as well as valuable references.

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