

Case of tuberculosis in a Defassa Waterbuck (*Kobus ellipsiprymnus defassa*)

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Summary

In the Gdańsk Zoo a case of a disease induced by *Mycobacterium bovis* was recorded in a twelve-year-old male Defassa Waterbuck. The main symptom of the disease was persistent cough. The animal died as a result of anesthesia administered for the purpose of carrying out an intradermal TB test. The autopsy showed an advanced stage of pneumonia with multiple disseminated, partially purulent, partially calcified tubercles in both lungs and mediastinal lymphadenitis. Samples of the lung tissue and lymph nodes were sent to a laboratory for examination. During bacterioscopy, acid-fast bacilli were recognized. The culture and genotype identification revealed that the infection had been caused by *Mycobacterium bovis* ssp. *bovis*.

Keywords: *Mycobacterium bovis*, Defassa Waterbuck, GenoType MTBC

Every zoological garden makes efforts to avoid diseases caused by acid-fast bacilli, pathogens causing chronic diseases in different species of mammals, as well as in people (4, 7, 11). Tuberculosis is one of the most dangerous diseases in zoo animals (5, 6, 8, 12). For that reason, animals susceptible to tuberculosis, especially all ruminants, are examined by the TB intradermal test before being transferred from one zoo to another. This is the best way to avoid the disease, but unfortunately it is not completely reliable.

The Defassa Waterbuck (fig. 1) is very popular in zoological gardens because of its attractive appearance. It is a large antelope with horns of considerable size. Males weigh between 200 and 300 kg, and females up

to 200 kg. The species naturally inhabits areas close to water in the south of the Sahara region.

Case report

A male Defassa Waterbuck was born in the Hannover Zoo in 1997. The following year the antelope was transported to the Świerkocin Safari Park. A few years later the animal was sent to the Zoological Garden in Gdańsk with the report that the TB skin test had been negative. In the Gdańsk Zoo the male was kept together with a female of the same species. Before joining the male, the female had undergone a TB skin test with negative results. The animals were kept in a separate enclosure and had no contact with other animals. After five years in the Gdańsk Zoo, the male began to cough, which was plainly correlated with deterioration in its general condition. A few weeks of treatment with three different antibiotics administered intramuscularly (penicillin, streptomycin, tetracycline) produced no result. For this reason a decision was made to examine the animal by the TB intradermal test (3). The antelope was immobilized with a combination of medetomidine and ketamine, and the tests were performed using bovine as well as avian tuberculin. The animal recovered from anesthesia without complications. Three days later it was immobilized in the same way as the first time (the same drug combination and doses) in order to read the results of the tests. The tests gave negative results since neither oedema nor inflammation was observed in sites where the tuberculins had been injected. Unfortunately, the male died during immobilization. The application of a reversal agent for medetomidine (atipamazole) gave no result.



Fig. 1. Defassa Waterbuck

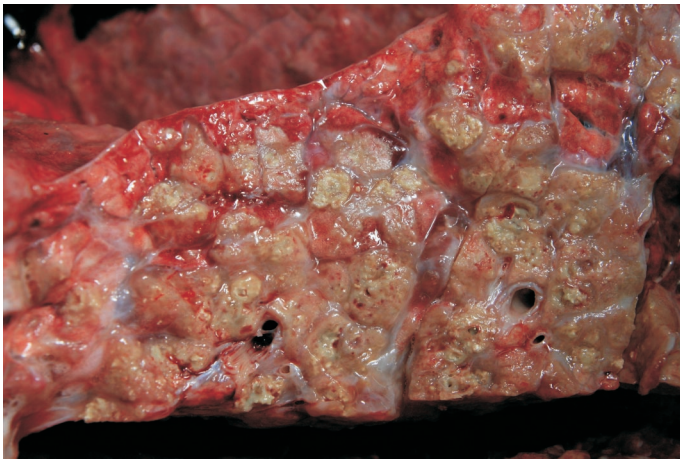


Fig. 2. Lesions in Deffassa's lung tissue

The autopsy showed mediastinal lymphadenitis and an advanced stage of pneumonia with multiple disseminated, partially purulent, partially calcified tubercles (fig. 2) in both lungs. The lesions were characteristic of tuberculosis. Samples of the lung tissue and lymph nodes were sent to a laboratory for examination.

A few days after the male's death, the female and a one-year-old male offspring were tested by the TB intradermal test. The result of the test was negative in the young animal but positive in the female: in the site of injection of the bovine tuberculin a clear oedema was observed (an increase in the thickness of the skin fold was 10 mm). The decision to euthanize both animals (the male offspring and the female) was taken. During the autopsies of both animals mesenteric lymphadenitis (without calcification) was found. The bacterioscopy of samples taken from the female revealed acid-fast bacilli.

Material and methods

Infectious material for laboratory investigation consisted of tissues of a Deffassa Waterbuck: lung slices and thoracic lymph nodes. Carefully viewed and fragmented, the tissues revealed the presence of typical tuberculous lesions. The imprints of lung slices and lymph nodes were stained with the Ziehl-Neelsen stain and examined for acid-fast bacilli. Tissue samples with changes were homogenized and decontaminated according to the Instruction of the Central Veterinary Officer (3) on the detection of *Mycobacterium bovis* in particular. The sediment was inoculated onto 4 Stonebrink's, 2 Loewenstein-Jensen's and 2 Petragani's slants and incubated at 37°C for up to 6 weeks with weekly reading. The material for genotype identification was the isolate from Stonebrink's slants. Bacterial DNA was extracted using a Dneasy Blood&Tissue Kit (QIAGEN, Germany). The typing of the species was performed by a commercial test, GenoType MTBC (HAIN Lifescience, Germany) (2, 6, 9, 10). This kit is based on the DNA STRIP technology and enables the genetic differentiation of the species belonging to the *Mycobacterium tuberculosis* complex on the basis of gyrase B gene polymorphisms. The hybridization and detection was carried out in a pre-warmed shaking water bath according to the manufacturer's protocol. The entire reaction occurred in membrane strips coated with specific probes. After drying, the strip pattern of stained bands cor-

responding to a specific DNA sample was evaluated by comparing it with the model supplied by the manufacturer.

Results and discussion

In a clinical examination, the Deffassa Waterbuck showed symptoms of infection of the respiratory system. Anatomical pathology examination suggested changes characteristic of tuberculosis only in lung tissues. Thoracic lymph nodes were enlarged and congested, without tubercles visible to the naked eye. Microscopic examination of the samples did reveal acid-fast bacilli only in lung preparations. After 6 weeks, an abundant growth of mycobacteria was visible on Stonebrink's slants. On four Stonebrink's slants where typical mycobacterium cultures had grown, a molecular method was used. On the other slants only a single colony of mycobacteria was visible. In all cases, the cultures that had been isolated were classified as *Mycobacterium bovis*. Genotype identification proved that infection was caused by *Mycobacterium bovis ssp. bovis*. The result showed that the decision to euthanize the female and its male offspring had been correct, since tuberculosis is a significant zoonosis that can spread to humans (1, 13). Considering the need to keep the highest sanitary standards in a zoological garden visited every year by hundreds of thousands visitors, it is impossible to tolerate the presence of such a pathogen (4).

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