Avian tuberculosis 
in a captive cassowary (Casuarius casuarius)

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Abstract

The paper describes avian tuberculosis in a captive bred cassowary. A two-and-a-half-year-old bird was obtained by a Polish zoo in 2010 from the Netherlands under conditions compliant with the recommendations of the European Association of Zoos and Aquaria. Despite being of small size for the age, the bird appeared healthy and showed no signs of the disease until the day when it was found recumbent in its pen. Later on it was euthanised due to lack of treatment possibilities. Pathological changes typical of avian tuberculosis were found in the liver and spleen. Mycobacterium avium ssp. avium was cultured from both organs.

Keywords: cassowary, avian tuberculosis, Mycobacterium avium ssp. avium, zoo.

Introduction

Mycobacteria include a group of over 140 atypical bacteria described as mycobacteria other than tuberculosis (MOTT) or non-tuberculous mycobacteria (NTM) (15). There is a continual increase in the number of identified mycobacteria species due to the development of molecular methods (23). Due to the constant presence of mycobacteria in the environment, people and animals are continually exposed to MOTT. Mycobacterial infection is an infectious disease which can affect all animal species: fish, reptiles, birds, and mammals (2, 4, 8, 10, 22). Mycobacterium avium, which in birds causes typical tuberculosis, is also pathogenic to immunodeprived humans and other animals, causing a disease known as mycobacteriosis.

Cassowaries belong to the superfamily which includes three species of land and flightless birds. They live in New Guinea and north-eastern Australia, and the islands have had them in included in the Red List of Threatened Species. One example for such a vulnerable species is the Australian Southern cassowary (Casuarius casuarius johnsonii) which is endemic to the north-western area of Queensland in Australia (1). The population of these birds is endangered due to habitat destruction, road kills, dog attacks, hand feeding, diseases, and cyclones (9).

Material and Methods

Case description. A two-and-a-half-year-old male bird was obtained by the Warsaw Municipal Zoological Garden in 2010 from the Netherlands under conditions compliant with the recommendations of the European Association of Zoos and Aquaria. From the beginning it showed stunted growth and minor anatomical abnormalities: a deformed jaw and deformation in the crop area, which was thought to be due to hand rearing and the character of the bird. The veterinarian attending the cassowary was informed of its unusual calm behaviour. There were no other symptoms such as lack of appetite or more solid faecal consistency. In few days, the bird started to behave normally. About a month later, the cassowary was found recumbent with breathing problems and was easily approachable by the staff. A clinical examination of the bird demonstrated that the oesophagus and crop seemed to be extended.
Ketamine (2 mg/20 kg) and xylazine (2 mg/kg) were used for sedation of the bird and then the animal was intubated and supported by inhalation anaesthesia using a mixture of oxygen and isofluorane (1.5%–2.5% support concentration). The lateral skin was incised and a thick, brown, amorphous mass was seen, which was not directly related to the oesophagus. Further examination showed that the mass consisted of coagulated blood. The changes were considered inoperable and the veterinarian decided to euthanise the bird.

**Microbiology.** Material from the liver and spleen was taken for microbiological analysis. The examination was performed in accordance with the OIE Manual (Chapter 2.3.6: Avian tuberculosis). The material was then homogenised in a 5% solution of oxalic acid. The resulting supernatant was removed and the pellet was washed twice with sterile physiological saline. The rinsed pellet was used for inoculation of culture media, which was three Stonenbrinck’s slants and three Petragnani medium slants, and incubated at 37°C.

**Microscopy.** The cultures from all slants were stained with Ziehl–Neelsen stain and examined for acid-fast bacilli.

**Identification.** The typing of the isolated *Mycobacterium* was performed by GenoType CM® (HAIN Lifescience, Germany). The test comprises 38 molecular models, 24 of which can be assigned to different species of mycobacteria, 10 patterns correspond to two or more species and 4 correspond to the patterns of *Mycobacterium* spp. or gram-positive bacteria with high G-C pair contents of the genome (16, 17, 18). The analysis consisted of three main stages: isolation of DNA from the isolated strain, amplification of genetic material by using biotinylated primers, and hybridisation. The results were shown on nitrocellulose strips in the form of dark bands arranged in a specific configuration. Comparison of strips with patterns included by the manufacturer led to their differentiation and the typing of the isolated *Mycobacterium*.

**Results**

Post-mortem examination did not reveal any changes in the respiratory or nervous systems, nor the pancreas. The liver was enlarged, brittle, and dark brown with numerous suppurative granulomas from 1 to 4 mm in size, which were observed on the surface of the organ and in the cross-section (Fig. 1). The spleen was of friable consistency, pale green to dark-red, and almost totally covered with granulomatous lesions (Fig. 2). The kidneys were enlarged and dark-red coloured. There were no changes in the mucosa of the crop or oesophagus. The stomach was correctly filled and gastric mucosa was not congested. Intestinal mesenteric lymph nodes were extremely enlarged to the size of ping-pong balls. The bursa of Fabricius was the same size.

After about 20 d, abundant growth of the *Mycobacterium* was observed on both media. Examination confirmed the presence of acid-fast bacteria in all preparations (Fig. 3). The results of genotyping showed that the isolated strain was classified as *Mycobacterium avium* spp. *avium*.

**Fig. 1.** The brittle, dark-brown liver with numerous suppurative granulomas
Discussion

Avian tuberculosis is an infectious disease of all species of exotic, wild, and domestic birds, caused by *Mycobacterium avium* (5, 7). It is a chronic debilitating disease that can persist for years in an infected flock. Initially clinical signs of infection are absent, with no changes in appetite or growth rates. As the disease progresses there is a gradual emaciation, with preserved appetite. The condition of the birds deteriorates and in many cases lameness and diarrhoea are observed. Birds die due to extreme exhaustion. The following
diseases should be considered in the differential diagnosis of avian tuberculosis: yersiniosis, avian pseudotuberculosis, coligranulomatosis, listeriosis, histomoniasis, aspergillosis, and cancer.

The literature data shows that we know a lot about viral and bacterial infections of fowl in Poland (3, 6, 11, 21). Wild birds living outside as well as inside zoos are also a subject of interest to many Polish research centres (12). Unfortunately, obtaining test samples from wild specimens is not as easy as from those kept on poultry farms. The subject of *M. avium* infections in poultry and wildfowl in Poland seems to be somewhat neglected. It should be emphasised that avian tuberculosis causes typical pathological changes in the bird’s liver and spleen, which is why the majority of fatal cases in zoos are rarely further investigated in the reference laboratory. This confirms the opinion that tuberculous lesions in the lungs are rare in cases of *M. avium* infection in birds (2). Numerous acid-fast structures represent a further positive result of microscopic examination and are sufficient to dispel any doubt in making the diagnosis (Fig. 3). Avian tuberculosis is typically a chronic disease, very frequently secondary to other health issues (20). Some species seem to be more prone to this disease, and the cassowary is a great example of a ground-feeding species that has more potential means of exposure to mycobacterium species, but which should be more resistant. Numerous studies show (2, 20) that exposure to tuberculosis infection correlates closely with the concentration of infectious particles and time of exposure. Therefore, birds remaining together in close and lasting contact are more exposed to infection, which obviously relates to birds living in pairs or sharing enclosures with pest species like sparrows, pigeons, *Corvidae*, or peacocks (free ranging in many zoos) that cannot be completely eliminated. Ante-mortem diagnosis includes PCR, ELISA, or intradermal tuberculin skin test. During clinical examination special care should be taken to examine the oral cavity for plaques that may be related to the disease. Birds that are positive should be culled due to their zoonotic potential.

The most common disease in the cassowary is avian tuberculosis (14). According to Pavlik et al. (13) there are also other mycobacterial infections like *Mycobacterium bovis* which cause the death of birds. However, the total reported number of cassowary deaths due to diagnosed diseases is very low (9). *M. avium* infection should be regarded as a candidate diagnosis in chronic weight loss and emaciation, although it is hard to notice in non-flying birds as the pectoral muscles are relatively small (20). Avian tuberculosis is a possible threat to zoo- and free-living populations of cassowaries. The role of the cassowary in the spread of *M. avium* and other types of tuberculosis complex infection should be considered in animal collections like zoological gardens.

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**References**
