Mycobacterioses of birds, also known as avian tuberculosis, are a long-known problem often occurring in Galliformes (13). In modern large-scale poultry farms, where birds are kept for a period of several to several dozen weeks, and the bedding is replaced after the end of each production cycle, mycobacterioses are extremely rare (29). However, the problem of mycobacterioses is still present mainly in free-range poultry, as well as in zoos and parks, where the birds have contact with soil contaminated with faeces, which can be a source of mycobacterial infections for many years (7, 14, 18, 21). Although ill birds are eliminated in reasonably managed parks and zoos, individual owners are often very attached to their animals and do not consent to euthanize the sick bird, but ask for treatment (4, 26). Unfortunately, treatment of mycobacterioses is long and costly, and its outcomes are uncertain (15, 24, 26). Bacteria belonging to the Mycobacterium avium complex (MAC) are intrinsically resistant to many antibiotics and antituberculosis drugs (30). There is also the risk that the bacteria may acquire drug resistance during treatment (5). The potential threat to the health of other animals and humans is also of considerable importance (13, 27, 30). To date, the authors have diagnosed three cases of peafowl (Pavo cristatus) mycobacteriosis caused by Mycobacterium avium, which is also pathogenic for birds (2, 20).

Material and methods

Birds examined. The material for the study were three live birds and one dead bird obtained at different times. The first bird (Peacock 1) was a five-year-old that had shown intensifying signs of dyspnoea, also at rest, and severely decreased appetite for approximately one month. The second case (Peahen 2) was a three-year-old with severe dyspnoea. During the procedure of obtaining a swab specimen from the trachea, the peahen expectorated approximately 2 ml of thick, mucous fluid that was subsequently tested for the...
The presence of *Mycobacterium*. The third case (Peacock 3) was a two-year-old originating from the same farm as Peahen 2 and was brought to the clinic 1.5 years after the death of the female. Its clinical signs were identical to those in the two previous cases. The fourth case (Peahen 4), submitted for necropsy, was under the age of 1 year. A sudden death due to a trauma was determined in that bird.

**Initial therapy.** Initially, symptomatic treatment was applied, consisting in subcutaneous administration of fluids (glucose 5% + sol. Ringeri Lactate + Duphalyte), enrofloxacine (20 mg/kg bw per day: Pefowls 1 and 2) or marbofloxacine (10 mg/kg bw per day: Peacock 3).

**Diagnostic imaging.** X-ray examination and tracheoscopy were conducted in Peacock 3. For general anaesthesia, butorphanol (Butomidor, Rihter Pharma AG) at 0.5 mg/kg bw i.m., and isoflurane (Aerrane, Baxter) at 5% for induction and 2% for maintenance, were used. An endoscopic trachea examination was conducted using a rigid endoscope NOPA XP 700/33. X-ray examination was conducted in Peacock 3 in lateral and dorso-ventral positions.

**Sampling material for tests.** Tracheal swabs were collected from all three live birds for a bacteriological test for mycobacteria, and faecal matter was collected for a parasitological test and bacterioscopy (Ziehl-Neelsen stain).

Peacock 3 was euthanised, and fragments of its organs (lungs, liver and spleen) were collected during necropsy. From the dead Peahen 4, fragments of caseous nodules located in the lungs and liver were sampled.

**Specimen staining and cultures.** Specimens stained by the Ziehl-Neelsen method were derived from tracheal swabs and organ impressions from Peacock 3 and Peahen 4. Tracheal swabs from Peafowl 1-3 and organ samples (lung, liver) from Peafowl 3 and 4 were cultured on the Löwenstein-Jensen PACT medium (BD, USA). Moreover, a tracheal swab from Peacock 1 and fragments of the lung and liver collected during necropsy from Peacock 3 were cultured on the BBL MIGIT medium in the BD BACTEC™ MGIT 960 system.

**DNA isolation and PCR amplification techniques and clustering analyses.** (CCG)4-based PCR for genotyping *M. avium* was performed according to the methodology described by Wojtasik et al. (34).

**PCR amplification techniques.** An amplification reaction for (CCG)4-based PCR using 50-N6(CCG)4 primers (N = A, T, C, or G) and gel electrophoresis was also performed according to a strict procedure described elsewhere (33, 34). The DNA products for (CCG)4-based primers ranged from 0.1 to 2.5 kbp. All gels were stained with ethidium bromide (1 lg ml⁻¹), visualized on a UV-transilluminator and photographed (Fc8800, Alphainnotech).

**Results and discussion**

**Clinical examinations.** In the three peafowl examined clinically, identical signs of dyspnoea (Fig. 1) and decreased appetite were observed. The general condition of the animals improved after treatment. Five months after the end of the two-and-a-half-month treatment of Peacock 1, the owner reported re-occurrence of dyspnoea signs, and despite his insistence on undertaking treatment again, euthanasia was recommended. Unfortunately, the owner did not submit the animal for necropsy.

Clustering analyses. (CCG)4-based PCR fingerprinting images were processed for further analysis with the BioNumerics software. The sizes of PCR products in each lane of agarose gels were normalized in reference to a 100-bp DNA size marker (Perfect 100-bp DNA ladder, EURX Ltd.) containing 13 fragments that ranged in size from 100 to 1,000 bp in 100-bp increments and additional fragments of 1.5, 2, and 2.5 kbp. Clustering analysis of the (CCG)4-based PCR band patterns was conducted with the BioNumerics software version 5.00 (Applied Maths), and a dendrogram was generated using average linkages. For differentiation analyses, the discriminatory index (DI) (10) was evaluated.

Tab. 1. Characteristics of the disease in the peafowl examined

<table>
<thead>
<tr>
<th>Peafowl no.</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Dyspnoea signs</th>
<th>Presence of mycobacteria in the tracheal swab stained by the Ziehl-Neelsen method</th>
<th>Positive result of the tracheal swab/sample culture</th>
<th>Presence of lesions in the trachea</th>
<th>Positive result of the internal organ cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>5</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>2</td>
<td>♀</td>
<td>3</td>
<td>+</td>
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<td>3</td>
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<td>+</td>
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<td>&lt; 1</td>
<td>–</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>+</td>
</tr>
</tbody>
</table>

Explanation: nt – not tested

Fig. 1. Signs of severe dyspnoea in Peacock 1
Treatment following diagnosis. In Peacock 1, in which an invasion by *Syngamus trachea* was also detected, ivermectin (Ivomec, Merial, France) at a dose of 200 µg/kg bw was administered i.m. After obtaining a positive result of tracheal swab culture, a targeted antimycobacterial treatment was undertaken. Enrofloxacin (Vetoflok, PLIVA Krakow) at a dose of 15 mg every 12 hours i.m. and clarithromycin (Klacid, Abbott, France) at a dose of 30 mg/kg bw every 12 hours p.o. were used, along with inhalations of enilkonazole (Imaverol, Jansen, Belgium), 2% solution in physiological saline, 25 min, 2 times a day.

Resting dyspnoea resolved after 10 days of treatment, while stress-associated dyspnoea (during immobilisation) resolved after the following 3 weeks. Throughout treatment, the peacock demonstrated decreased or selective appetite.

In all peafowl examined, improvement was obtained after supportive and antibiotic therapy. In Peahen 2, death occurred after approximately 3 weeks, but the enrofloxacin treatment lasted only 1 week. Peacock 3 lived for approximately 1 month after the first examination and received marbofloxacin throughout that period, which induced a short-term improvement. The owners submitted the animal for further diagnostics after that time had passed. The results of additional examinations (X-ray and endoscopy) were the basis for euthanasia.

Diagnostic imaging. Diagnostic imaging was conducted only in Peacock 3. In the lower section of the trachea of Peacock 3, a nodule blocking ¾ of the tracheal lumen was detected (Fig. 2). The nodule was visible in X-ray images (Fig. 3). Moreover, X-ray revealed an opaque mass of 3 cm × 1.5 cm in the mediastinum (Fig. 3) and a mass of approx. 4 cm in diameter in the posterior part of the lung (Fig. 4). In the body cavity, enlargement of the spleen to 5 cm × 2.5 cm was observed (Fig. 4).

Microscopic examination. In the microscopic specimen of faeces from Peacock 1 stained by the Ziehl-Neelsen method, a moderate number of acid-fast bacteria were found.
In Peafowl 2 and 3, no acid-fast bacteria were found either in the tracheal swab or in the faeces specimen.

**Microbiological examination.** In Peacock 1, a positive result was obtained for the direct sample from the tracheal swab, and growth of *Mycobacterium avium* subsp. *avium* was observed on culture media, both before treatment and after three weeks of treatment. However, negative results for swabs were obtained two weeks and two months after the end of treatment. In the case of Peahen 2, mycobacterial growth on the Löwenstein-Jensen medium was obtained. The tracheal swabs taken from Peacock 3 did not yield positive results in the bacterioscopic examination and growth on the Löwenstein-Jensen medium. Growth of *Mycobacterium avium* subsp. *avium* from this individual was obtained only in cultures of the lung and the liver. With regard to the necropsy material from Peacock 4, a positive result was obtained for culture and bacterioscopy.

**DNA isolation and PCR amplification techniques and clustering analyses.** The highest similarity was demonstrated by examining the strains of *Mycobacterium avium* subsp. *avium* from Peahen 2 and Peacock 3 originating from the same farm. The similarity of the strain derived from Peacock 1 was high as well. However, the *Mycobacterium avium* subsp. *paratuberculosis* strain isolated from Peahen 4 had only approx. 38% similarity with the other isolates (Fig. 5).

In peafowl, signs of severe dyspnoea not associated with mycobacteriosis can be caused by aspergillosis and other bacterial (mycoplasmosis) and viral infections, as well as by advanced syngamosis and presence of foreign bodies (25, 27, 28). It is also possible to isolate several pathogens in one case (21). Signs identical to those of mycobacteriosis have been seen by the authors in peafowl with avian pox with respiratory involvement or caseous lesions in the posterior larynx (Syrinx), from which *Enterobacter* sp. was isolated. The difference in the clinical course of respiratory avian pox and mycobacteriosis was the more rapid development of the former and a larger number of concurrently affected animals. In the case of *Enterobacter* infection, despite clinical signs identical to those in Peahen 2, no typical lesions were found in X-ray examination. As can be inferred from the cases described, mycobacteriosis cannot always be reliably diagnosed in live birds, even those with advanced clinical signs. In the intravital diagnostics of mycobacterioses, it is particularly useful to take biopsies directly from the affected organ during an endoscopic examination (8). However, in a study by Saggese et al., a positive result of a biopsy was obtained in only 3 out of 16 infected birds (26).

No information on the treatment of mycobacterioses in Galliformes has been found in the available literature. Suspected birds, e.g., those showing a positive tuberculin sensitivity test, were eliminated from the farm (3). Vaccines against mycobacterioses have been developed and show a good effectiveness, but are not marketed (6, 16). Treatment has been attempted in domestic birds, including collared doves and parrots (14, 26). It is recommended to isolate treated birds from the flock for at least 2 years and to conduct bacteriological examinations every 6 to 12 weeks (23). Effective treatment of mycobacterioses has been conducted in accompanying animals, such as cats.
dogs and ferrets (1, 10, 17, 19, 22, 31, 32). However, treatment of mycobacterioses is conducted mainly in humans. AIDS patients are a group in whom mycobacterioses are the most serious problem. In humans, treatment with two or three medicines should last up to 12 months (12). Treatment of mycobacterioses in ornamental birds of the Galliformes genus is possible, but even a 4-month therapy may be insufficient (14, 24). On the other hand, a 6-month therapy can produce satisfactory results (26). Some authors recommend that treatment of mycobacterioses in birds should be continued for as much as 1 year. As demonstrated by case reports, treatment in other accompanying animals usually lasted from several weeks to over a year (1, 5, 11, 31). Animals treated for mycobacterioses would often die after or during treatment for reasons other than the infectious disease (17).

In practice, administering drugs over such a long period and maintaining an appropriate sanitary regime requires discipline and responsibility on the side of animal carers. Irregular administration of drugs may lead to the onset of bacterial resistance. Resolution of clinical signs, as in the case of Peacock 1, does not guarantee that the disease has been cured. Therefore, we do not recommend leaving treatment to an owner who may not be aware of the responsibility and risk.

References


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