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Avian mycobacteriosis in an individual of the endangered Mauritian Pink pigeon (*Nesoenas mayeri*) species: a case report

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ABSTRACT: Avian mycobacteriosis was diagnosed in one female Mauritian Pink pigeon (*Nesoenas mayeri*) from a zoological collection. The bird displayed clinical symptoms of apathy, loss of body weight, ruffled feathers and diarrhoea. Necropsy revealed nodular lesions in the internal organs. *Mycobacterium avium* subsp. *avium* was isolated and also directly detected using qPCR in the female's liver. Avian mycobacteriosis may pose a threat to efforts directed towards conservation of this endangered species.

Keywords: *Columba*; conservation; dove; tuberculosis

Avian mycobacteriosis is a chronic infectious disease of both domestic and wild birds characterised by a long incubation period and non-specific clinical symptoms, e.g. wasting, loss of weight to emaciation and diarrhoea; and the occurrence of microscopic and/or macroscopic granulomatous inflammatory lesions (tuberculous lesions) in various organs. Several species of mycobacteria can be causative agents of this disease. *Mycobacterium avium* complex members (*M. a. avium* and *M. a. hominissuis*) have been frequently isolated from infected birds (Shitaye et al. 2009; Kriz et al. 2011). The Mauritian Pink pigeon (*Nesoenas mayeri*) is an endemic columbid species which has survived on the Island of Mauritius in the western Indian Ocean. Although critically endangered by extinction in the early 1990's with a mere 10 individuals remaining, the wild population of Pink pigeons gradually increased to more than 440 individuals in 2011 due to an intensive conservation program. Key components of the program include captive breeding both in Mauritius and at the Jersey Zoo and the release of birds into the wild. Further captive subpopulations of Mauritian Pink pigeons have been established in many zoological collections around the world. Avian mycobacteriosis caused by *M. a. avium* has been occasionally observed in various columbids (Saggese et al. 2008; Kriz et al. 2011); however, according to the available literature it has not yet been described in Mauritian Pink pigeons.

Case description

A three year and six months old female Mauritian Pink pigeon from a zoological collection in the Czech Republic was presented to a veterinarian because of impaired body condition. During the first presentation, the bird showed inappetence, apathy and ruffled feathers. Marbofloxacine 10 mg/kg *i.m.* SID (intramuscular injection, single in a day dosage), (Marbocyl 20 mg/ml inj., Vetoquinol s.r.o., Nymburk, Czech Republic) was administered for

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one week and the clinical status of the bird gradually got better. However, the condition of the bird worsened over the subsequent ten days after the cessation of the therapy. At the second presentation the bird weighed 220 g (normal body weight of adult female pink pigeon is about 315 g), and displayed apathy, inappetence, and ruffled feathers. Parasitological examination of faeces was negative. Antimicrobial therapy using marbofloxacin was started again but no response was observed over the subsequent days.

A firm nodule (2 cm in diameter) was found by palpation on the right side of the breast bone under the breast muscle. Radiographical examination confirmed the presence of a radiolucent lesion on the right side of the breast bone (Figure 1). Clinical haematology performed using standardised methods revealed leucocytosis (39.6×10^9/l) caused by heterophilia (30.8×10^9/l) and monocytosis (7.1×10^9/l). Markedly increased activities of creatine phosphokinase (CPK; > 2000.66 IU/l), aspartate aminotransferase (AST; 584.9 IU/l) and lactate dehydrogenase (LDH; >901.65 IU/l) were found during biochemical analysis of blood (Fuji Dry-Chem 4000i, Fujifilm Europe GmbH, Prague, Czech Republic). The bird was put on a therapy of Meloxicam 1 mg/kg *s.c.* SID (subcutaneus injection), (Metacan 5 mg/ml inj., Boehringer Ingelheim Vetmedica GmbH, Ingelheim am Rhein, Germany) and phenoxymethylpropionate 10 mg/kg *i.m.* SID (Hepagen inj., FATRO S.p.A. – Industria Farmaceutica Veterinaria, Ozzano Emilia, Bologna, Italy); however, on the eighth day of therapy the health condition worsened dramatically with the occurrence of diarrhoea and the bird died.

The necropsy revealed a grey-to-yellowish nodular lesion growing from the body cavity through the breast bone. Multiple grey-to-yellowish nodular lesions of 1 to 15 mm were seen in the liver (Figure 2), as well as in the kidneys and the intestinal wall. Samples were collected from all these organs and were fixed in buffered 10% neutral formalin, dehydrated and embedded in paraffin wax. The samples were then sectioned on a microtome and stained with haematoxylin and eosin and according to Ziehl-Neelsen (ZN).

The sample of liver (1 g) was homogenised and decontaminated using the HCl-NaOH method according to a procedure described previously (Fischer et al. 2000). Each decontaminated sample was inoculated onto Herrold's egg yolk medium and Löwenstein-Jensen with sodium pyruvate in duplicate and then incubated at 37 °C. Suspected colonies were checked using ZN staining and then subcultured on Herrold's egg yolk medium. Obtained colonies were again stained according to ZN and subsequently identified using a multiplex PCR method described previously (Moravkova et al. 2008).

Direct detection and quantification of mycobacterial DNA from the liver sample was performed using an isolation procedure with a commercially available kit and the DNA was detected and quantified using a triplex quantitative real time PCR previously described by Slana et al. (2010).

Multiple foci of chronic nodular granulomatous inflammation with central necrosis and macrophages in the centre and lymphocytes on the periphery were seen in the tissue sections. Multiple giant multinucleated cells of the Langhans' type

Figure 1. Radiolucent lesion (arrow) on the right side of the breast bone

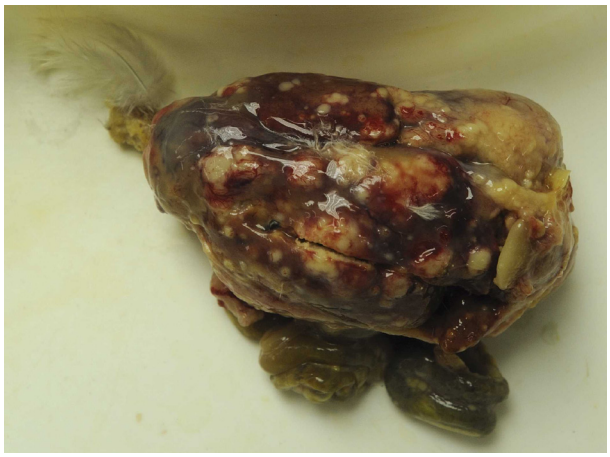

Figure 2. Multiple grey-to-yellowish nodules in the liver and intestine

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were present on the periphery of the lesions. The structure of all the organs was markedly affected by the multiple lesions. ZN staining showed the presence of acid-fast bacilli (AFB).

Culture of liver yielded a *M. a. avium* isolate and *M. a. avium* was found in numbers of 5.16×10^7 cells per one g of liver sample, as determined using qPCR.

DISCUSSION AND CONCLUSIONS

Tuberculous lesions in the liver, as well as the spleen and intestine have been frequently detected in columbids with *M. a. avium* infection/disease, although many other tissues and organs have been affected occasionally, e.g., lungs, air sacs, kidneys, gonads, bone marrow, etc. (Saggese et al. 2008; Kriz et al. 2011). The localisation of tuberculous lesions in the female bird described here suggests an oral route of infection with subsequent dissemination of the causative agent through the gastrointestinal tract into the parenchymatous organs.

Typical histopathological pictures of granulomatous inflammatory reaction and AFB were observed in the liver, as well as intestine and kidneys. The nodule growing through the breast bone of the bird was most probably of the same aetiology but unfortunately, it was not sent for histopathology or culture. A tuberculous lesion growing through the breast bone has not been reported in columbids yet. Most probably, the primary focus was the serosal surface in the body cavity after dissemination of *M. a. avium* from the intestine.

As there were no available reference values for interpretation of clinical haematology and biochemistry of Mauritian Pink pigeons, more general reference data from birds of the order Columbiformes had to be used (Gayathri et al. 2004; Prinzinger and Misovic 2010). Thus it was necessary to perform very careful interpretation.

Leucocytosis with heterophilia and monocytosis has been associated with avian mycobacteriosis in birds; e. g., Hawkey et al. (1990) made the same observation in *M. a. avium*-infected domestic fowl and cranes and Saggese et al. (2008) in infected ring-neck doves. Monocytosis has been considered as a significant marker of avian mycobacteriosis in birds (Hawkey et al. 1990). Increased activities of CPK and LDH were most probably associated with muscular atrophy due to emaciation and the nodule growing from the body cavity exerting pressure on the breast muscle. Increased levels of AST can be explained by the destruction of the liver parenchyma by multiple tubercles.

The survival of the wild population of Mauritian Pink pigeons is endangered by several factors, e.g., introduced predators, shortage of native habitats and food, regular occurrence of cyclones, inbreeding and infections (Swinnerton et al. 2004). The negative impact of trichomoniasis (*Trichomonas gallinae*) as well as leucocytozoonosis (*Leucocytozoon marchouxi*) on the wild Pink pigeon population on Mauritius is well documented (Swinnerton et al. 2005; Bunbury et al. 2008).

According to available literature, avian mycobacteriosis has yet to be reported either in captive or in wild Mauritian Pink pigeons. Thus, it is not possible to draw any conclusions regarding the significance of *M. a. avium* infection for this species. However, the insidious character of the disease accompanied by a lack of both rapid and reliable *ante-mortem* diagnostic methods and effective therapy may pose a threat to efforts made in the framework of the conservation program for Mauritian Pink pigeons.

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