Ante mortem diagnosis of mycobacterial infection by liver biopsy in a budgerigar *(Melopsittacus undulatus)*

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Summary

Avian mycobacteriosis is an important disease which affects exotic, wild and domestic birds. The disease is most commonly caused by the ubiquitous soil and water organisms *Mycobacterium avium* sp. *avium* and *Mycobacterium genavense.* Mycobacteriosis should be considered in the differential diagnosis of any pet bird with chronic disease characterized by weight loss, an inflammatory leukogram and abdominal enlargement. Ante mortem diagnosis of avian mycobacteriosis in birds remains difficult. The present case report describes that even in very small species such as a budgerigar the investigating of a biopsy sample is an efficient way to rule out or confirm the disease in cases where other methods fail to be conclusive.

Keywords: mycobacteriosis, diagnosis, liver biopsy

Diagnose einer Mykobakterieninfektion durch Leberbiopsie bei einem Wellensittich *(Melopsittacus undulatus)*

Die aviäre Tuberkulose gehört zu den wichtigen Erkrankungen der Zier-, Wild- und Wirtschaftsgeflügel und wird durch die ubiquitär vorkommenden Erreger *Mycobacterium avium* sp. *avium* und *Mycobacterium genavense* verursacht. Krankheitssymptome wie Gewichtsverlust, das Vorliegen eines entzündlich veränderten Blutbildes und eine Vergrösserung des Zoeloms können auf eine aviäre Mykobakteriose hinweisen. Die Diagnose der Vogeltuberkulose am lebenden Tier, insbesondere bei kleinen Spezies, erweist sich nach wie vor als schwierig. Eine sichere Diagnose kann durch Biopsie der veränderten Organe mit anschliessender Ziehl-Neelsen-Färbung gestellt werden. Diese Methode, welche im folgenden Fallbericht eines Wellensittichs dargestellt wird, erweist sich als effizient wie praktikabel und ist anderen, zeitaufwendigeren und teureren Untersuchungsmöglichkeiten wie z. B. der Kultur oder Serologie überlegen.

Schlüsselwörter: Mykobakteriose, Diagnostik, Leberbiopsie

Introduction

Mycobacteriosis is a common infection in birds, especially in psittacine birds (Ritchie, 1988). Albicker-Rippinger and Hoop (1999) found that of the 1866 psittacine birds submitted for post mortem 15% had a mycobacteriosis. Primary organisms causing mycobacteriosis in birds are *M. avium* and *M. genavense.* The clinical symptoms are not specific. Chronic weight loss, diarrhoea, dyspnoea, lameness and poor feathering may be seen. An ante mortem diagnosis is difficult especially for the practitioner. Several diagnostic methods may need to be combined to make a reliable ante mortem diagnosis of mycobacteriosis. Haematology may indicate a mycobacteriosis, when leucocytosis with monocytosis is present. However, other common avian infectious diseases such as aspergillosis and chlamydophilosis commonly result in similar haematological findings. The acid-fast mycobacterium may be detected in faeces after staining with Ziehl-Neelsen, but excretion is intermittent and the method is not reliable. Radiography and ultrasound may indicate a mycobacteriosis by revealing focally increased density in long bones or by thickening of the intestinal mucosa, but absence of such symptoms does not rule out a mycobacteriosis. Serological tests are available as well, but only for a limited number of avian species e.g. waterfowl, domestic fowl, raptors and cranes (Hawkey et al., 1990).

The present case intends to present an additional diagnostic method, which is not species specific. In our experience, examination of a biopsy from liver for Mycobacterium is an efficient way to confirm the disease, and biopsy may also be performed in small birds.

History and diagnostic procedure

A 4-year-old male budgerigar *(Melopsittacus undulatus)* from an aviary was presented to the Division of Zoo

 DOI 10.1024/0036-7281.149.06.273

Animals, Exotic Pets and Wildlife of the University of Zurich with a history of an enlarged coelom. Upon initial observation, the bird appeared in good general condition. Results of physical examination revealed normal body weight (48 g) and a mildly distended abdomen. Blood was collected and submitted for blood cell count and blood chemistry. A marked leucocytosis (121.6×10^3 leucocytes/µl, reference range 3.0×10^3 to 10.0×10^3 leucocytes/µl (Fudge, 1999)) and monocytosis (monocytes 18 per cent, reference range 0 to 2 per cent (Fudge, 1999)) was noted. Erythrocytes, haemoglobin, PCV, glucose, uric acid, total protein, calcium and phosphorus were within the normal range.

Whole body radiographs were obtained. Lateral and ventrodorsal pictures were made using Fuji Film® IP cassette type C (18 cm × 24 cm), 40 kV and 5.00 mAs with small focus. In addition, contrast X-rays were obtained 60 minutes after application of barium sulphate (20mg/kg p.o). On the plain radiographic views loss of the liver waist was noted and the grit filled ventriculus was displaced caudally. Contrast views revealed severe hepatomegaly and confirmed the caudal dislocation of the ventriculus (Fig.1a, b). No thickening of the mucosa of the small intestine would be observed.

For further diagnostic evaluation, ultrasonography of the coelomic cavity was performed. The budgerigar was anaesthetised using isoflurane (5% isoflurane for induction, 2–2,5% isoflurane for maintenance) and restrained in dorsal recumbency with legs extended caudolaterally. Warmed heat pads were placed under the bird to decrease heat loss. A ventromedian approach between the xiphoid process and the pubic bone was used for the sonographic examination with a 5 to 8 MHz micro convex transducer (Philips ATL HDI 5000) with a contact surface of approximately 0.7 × 2.7 cm. The liver was generally enlarged with hyperechogenic parenchyma and blunt margins (Fig. 2).

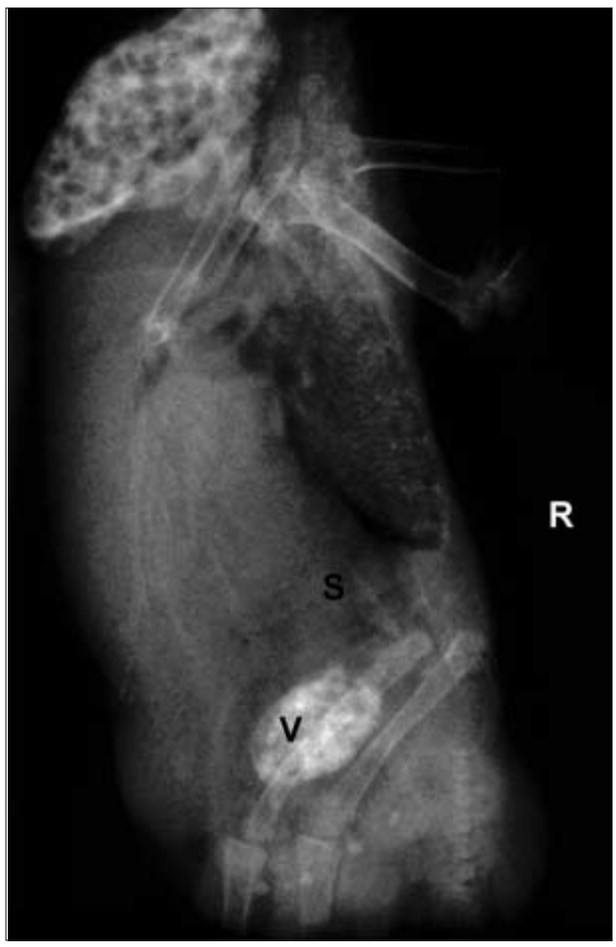

Figure 1b: Lateral contrast radiograph of the same budgerigar. Note the soft tissue structure (S) filling the coelom. The caudal thoracic air sacs are barely identifiable. The ventriculus (V) is displaced caudally.

Figure 1a: Ventrodorsal view of a contrast study of a budgerigar (Melopsittacus undulatus) with enlargement of the liver and the crop. Note the caudally displaced ventriculus (V) and lost of liver waist.

Diagnosis

Based on these findings, severe leucocytosis, monocytosis and hepatomegaly, an infection with *Mycobacterium* spp. was suspected. Differential diagnosis included infectious hepatitis due to *Chlamydophila psittaci,* neoplasia and lipidosis. Ziehl-Neelsen staining of cloacal smears was negative for acid-fast rods. To confirm the presumptive diagnosis of mycobacteriosis, a liver biopsy was obtained. The budgerigar was treated with 10 mg/kg Vitamin K i.m. (Konakion®MM, Roche, Switzerland) prior to surgery. Perioperative analgesia was achieved using butorphanol (1 mg/kg i.m., Morphasol®, Dr. E. Graeub AG Bern, Switzerland). Anaesthesia was in-

Figure 2: Transverse ultrasonographie image (median approach) of the coelom of the same budgerigar. Note the hyperechogenic liver tissue (L).

duced and maintained identically as for the ultrasound examination. The bird was positioned in dorsal recumbency. After surgical preparation, a ventral midline incision (approximately 1 cm) extending from the caudal border of the sternum was made. The liver was located and biopsies from two different locations were collected using 5 French biopsy forceps. The wound was closed routinely and the bird's recovery was uneventful.

The histological examination of the two biopsies taken from the liver revealed multifocal granulomatous hepatitis with acid-fast rods. A diagnosis of avian mycobacteriosis was made. Due to poor prognosis and zoonotic potential, euthanasia of the bird was proposed to the owner. The owner wanted to keep the bird alive without medication until its state of health deteriorates. Five weeks later, the bird was euthanized and submitted for post mortem examination. On gross necropsy, multiple white masses were noted throughout the enlarged liver. Histopathologic findings showed a multifocal granulomatous chronic hepatitis, enteritis, pneumonia, myocarditis and nephritis with acid-fast rods.

Discussion

Clinical signs of mycobacteriosis in birds vary widely. Common presenting symptoms of mycobacteriosis include weight loss, depression, anorexia, diarrhoea, dyspnoea, lameness and poor feathering. On physical examination emaciation, abdominal distension e.g. due to hepatomegaly, subcutaneous and conjunctival masses might be found (Korbel et al., 1997; VanDerHeyden, 1997). Granulomas are seldom seen and do not calcify (VanDerHeyden, 1997; Tell et al., 2001). In the present case a distinct hepatomegaly was seen on radiography and ultrasonography. In avian mycobacteriosis a mild to moderate hypochromic, microcytic anaemia (Hawkey et al., 1990) and a marked leucocytosis due to heterophilia and/or monocytosis are common findings (Hawkey et al., 1990; VanDerHeyden, 1997; Tell et al., 2001). Plasma biochemistry values are generally unremarkable. In the described case a marked leucocytosis with heterophilia and monocytosis were the only changes. Ideally acid-fast staining (Ziehl-Neelsen) of cloacal smears could allow a diagnosis. Because faecal shedding of mycobacterium organism may be very low and intermittent, mycobacterium organism can easily be missed. A positive result requires the presence of approximately 104 bacteria/g of faeces (Gerlach, 1994). In the present case Ziehl-Neelsen staining of cloacal smears was negative for acid-fast rods. Biopsies of affected tissue remain the most accurate way of diagnosing avian mycobacteriosis (Ritchie, 1988). Nevertheless, it should be kept in mind that *Nocardia* spp., very rare in birds, and *Arcanobacterium* spp., a component of the autochthonous flora in birds, stain similar to *Mycobacterium* spp. (Gerlach, 1994) with Ziehl-Neelsen, but compared to mycobacteria *Nocardia* spp. and *Arcanobacterium* spp. stain positive with Gram's stain (Barnes, 2003). Microscopic examination of biopsy tissue is a reliable, cheap and rapid procedure to detect acid-fast bacilli. In clinically ill birds a moderate to high concentration of mycobacteria are found in liver, spleen and intestines (Tell et al., 2003). However, collection of tissue samples is an invasive procedure in the live bird. Laparoscopy may be a useful technique for identifying mycobacterium lesions on the serosal surfaces of organs and allows biopsy of tissues (e.g. of the liver) (Lumeij, 1994; Tell et al., 2001; Kearns, 2003). But laparoscopy requires expensive equipment and in small birds such as a budgerigar may not be the method of choice for liver biopsy sampling. If endoscopy is unavailable or contraindicated due to marked abdominal enlargement, biopsy of liver tissue can be taken by laparotomy. In the anaesthetized bird a keyhole incision is made just caudal to the xyphoid to visualize the liver. Liver tissue can then be sampled using biopsy forceps. Coagulopathies have not been reported in infected birds and bleeding after biopsy has not been a problem (VanDerHeyden, 1997). Nevertheless, the possibility of severe haemorrhage secondary to liver congestion should be considered prior to biopsy in cases showing symptoms indicative of cardiac disease such as apathy, dyspnoea and abdominal distension due to ascites (Krautwald-Junghanns et al., 1999). Furthermore, caution should be taken when performing biopsies in birds that have prolonged bleeding time after blood collection (Lumeij, 1994). The present case demonstrates that the analysis of a liver biopsy may allow the diagnosis of mycobacteriosis where other extensive methods fail. The sampling does not require expensive equipment and may be performed quickly.

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Received: 20 July 2006
Accepted: 20 September 2006