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

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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 chlamyophilosis 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...
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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).

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-
 andra a mild to moderate hypochromic, microcytic radiography and ultrasonography. In avian mycobacteriosis, a distinct hepatomegaly was seen on the present case, but calcify (VanDerHeyden, 1997; Tell et al., 2001). Granulomas are seldom seen and do not calcify (VanDerHeyden, 1997). Nevertheless, it should be kept in mind that Mycobacterium 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 Mycobacterium 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 xiphoid 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.

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

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).
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References


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Received: 20 July 2006
Accepted: 20 September 2006