Avian tuberculosis in a captured Ruppell's griffon vulture (*Gyps ruppellii*): a case report

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ABSTRACT: Avian tuberculosis was diagnosed in a captured female Ruppell's griffon vulture (*Gyps ruppellii*) with granulomatous splenitis and hepatitis. At necropsy, whitish to yellow nodules of various sizes were found in the spleen and liver, and fibrinous coelomitis was present in the body cavity. Histopathologically, the granulomas appeared to be typical of avian tuberculosis. In some granulomas, necrotic centres surrounded by a variable layer of palisading epithelioid macrophages and multinucleated giant cells with variable admixture of lymphocytes and plasma cells were present. Signs of mineralization in granulomas were not observed. Using Ziehl-Neelsen staining the presence of acid-fast bacilli was demonstrated in organs affected by granulomatous inflammation. A diagnosis of *Mycobacterium avium* subsp. *avium* infection was confirmed by culture and quantitative Real-Time PCR examination for the presence of specific insertion sequences for avian tuberculosis (IS*901* and IS*1245*) in the liver and spleen.

Keywords: granuloma; mycobacteriosis; *Mycobacterium avium* complex; raptor; vulture; zoonosis

Avian tuberculosis is an important worldwide infectious disease that affects domestic or wild birds as well as exotic species (Lumeij et al., 1981; Piechocki, 1981; Schroder, 1981; Hoop et al., 1996; Tell et al., 2001; Jones, 2006; Shitaye et al. (2008a,b); Pate et al. (2009); Kaevska et al. (2010); Kriz et al. (2010); Witte et al., 2010). The disease may be present in a variety of clinical forms with most organ systems being affected. According to various authors, avian tuberculosis has been reported as the cause of death in 1 to 30% of raptor cases examined *post mortem* (Greenwood, 1977; Lumeij et al., 1981; Smit et al., 1987; Hoop et al., 1996; Morishita et al., 1998; Tell et al., 2004; Millan et al., 2010). The highest numbers of cases are diagnosed in captured birds from zoological gardens (Lumeij et al., 1981; Schroder, 1981; Tell et al., 2001; Jones, 2006). The occurrence of avian tuberculosis in zoological gardens represents a serious risk to the public's health not to mention the potential threat to other species. Interestingly, there is no report in the literature documenting avian tuberculosis in a vulture. The present report details a case of avian tuberculosis in a captured Ruppell's griffon vulture (*Gyps ruppellii*) caused by *Mycobacterium avium* subsp. *avium* (*M. a. avium*).

Case description

A free-living female Ruppell's griffon vulture (*Gyps ruppellii*) of unknown age was captured in Eastern Africa (2007) and bought by a zoological garden in the Czech Republic, where it spent two years until its death in October 2009. In October 2009, the vulture was presented with clinical signs

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of disorganized coordination of movement, weakness and cachexy. The feathers in the surrounding of the cloacal opening were stained by faeces. After examination, the vulture was treated with *intra muscular* systemic antibiotics and subsequently using a probe was fed with a mixture of raw ground meat and eggs into the crop. The vulture did not survive through to the end of the second day of treatment. The carcass was sent to the Institute of Pathology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic for an autopsy.

On gross examination, the vulture was judged to be cachectic and dehydrated and in very poor health status, as evidenced by a prominent keel and sinking of the eyes in the orbits. Prominent muscular wasting and loss of subcutaneous and cavitary fat was evident, as were discolouration and worn damaged edges of the feathers. There was mild subcutaneous oedema on the ventral part of the body in the cloacal region. The spleen was markedly enlarged and deformed by the presence of multiple firm whitish to yellow nodules, ranging in size from 0.1 to 1.5 cm in diameter. Nodules were often confluent; on cut section, some of them where seen to contain a centrally located necrotic mass (Figure 1). The liver was diffusely enlarged with rounded edges and multiple whitish miliary nodules were present in the parenchyma (Figure 2). Hyperaemia was also present in the same organ. In the body cavity, mostly between intestinal loops, there was a moderate amount of fibrinous exudate and the serosal surfaces of the intestine wall adhered to one another. Other pathomorphological findings included hyperaemia in the kidneys and lungs and dilatation of the right heart ventricle.

Subsequently, tissue samples (heart, lungs, trachea, liver, spleen, kidneys, stomach, intestines, and brain) were collected for histopathological examination. Samples were fixed in buffered 10% neutral formalin, dehydrated, embedded in paraffin wax, sectioned on a microtome at a thickness of 4 μm, and stained with hematoxylin and eosin (H&E) and Ziehl-Neelsen (ZN) for the detection of acid-fast bacilli in tissues. The liver and spleen were taken for culture and quantitative Real-Time PCR examinations.

Histopathological examination of the spleen revealed multiple foci of granulomatous inflammation. Smaller granulomas contained only epithelioid macrophages. Several larger granulomas also consisted of caseonecrotic centres surrounded by palisading epithelioid macrophages and multinucleate Langhans' giant cells. These granulomas were surrounded by variable amount of lymphocytes and plasma cells (Figure 3). At the periphery of the lesions, fibroblasts were occasionally present. There were no signs of mineralization in the granulomas. Histopathological examination of the liver revealed small multiple disseminated granulomas containing predominantly epithelioid macrophages, and mild lymphocytic infiltration lining macrophages. In several granulomas, central caseous necrosis was present (Figure 4). The liver granulomas also showed no signs of mineralization. ZN staining of the spleen and liver revealed large numbers of short rod-shaped acid-fast bacilli located centrally within the caseous necrosis present in granulomas

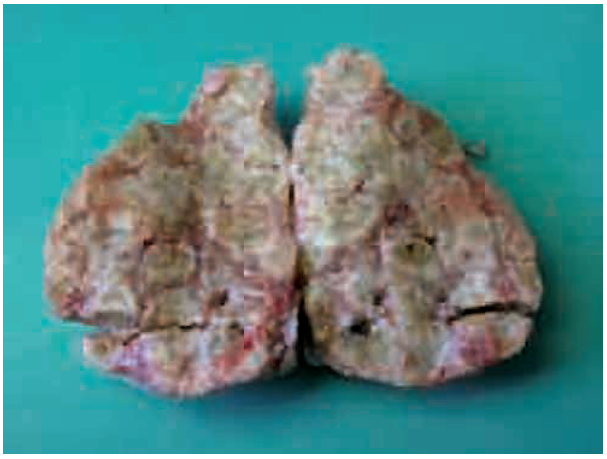

Figure 1. Spleen; vulture. Multiple coalescent whitish to yellow granulomas visible on cut surface

Figure 2. Liver; vulture. Markedly enlarged liver with rounded edges and presence of multiple miliary granulomas in the parenchyma

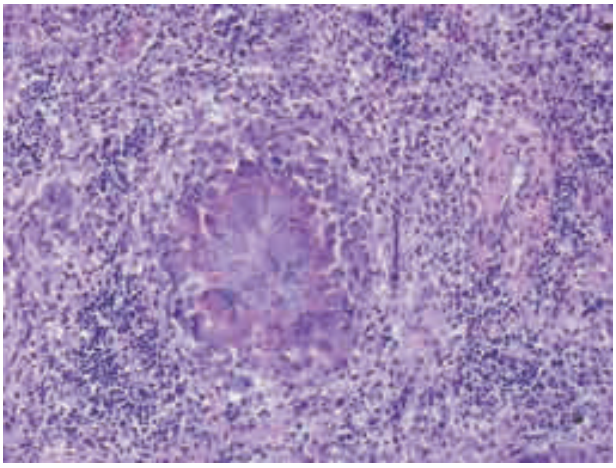

Figure 3. Spleen; vulture. **Granuloma with central necrosis** lined by epithelioid macrophages and lymphocytes on the periphery, HE, 400×

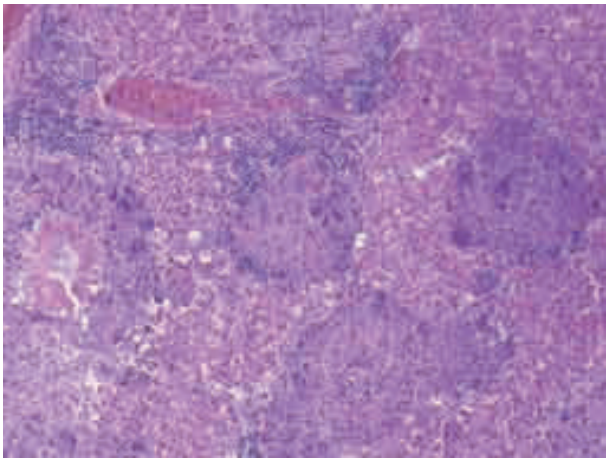

Figure 4. Liver; vulture. Multiple granulomas in the parenchyma, granuloma on the left with central necrosis, HE, 200×

and also on the periphery of the lesions (Figure 5). The acid-fast bacilli were localized free in the caseonecrotic mass and also inside the cytoplasm of macrophages (Figure 6). Fibrinous exudate made up of precipitated fibrin fibres and a moderate amount of heterophils was present on the serosal surface of the intestinal wall, as well as on the wall of body cavity. There were no granulomas present in the intestine. Microscopically, no other organs exhibited a granulomatous inflammatory reaction or any pathological lesions (except for the hyperaemia of organs and dilated right heart ventricle) and ZN staining was negative in all these tissue sections.

The liver and spleen samples were examined by quantitative Real-Time PCR (Slana et al., 2010), which confirmed the presence of IS*901* and IS*1245* in both organs. The number of IS*901* and IS*1245* copies in 1 g of tissue samples were as follows: for liver 3.24×10^{11} and 5.98×10^{11}, respectively, and for spleen 6.95×10^{10} and 1.17×10^{11}, respectively. Both tissue samples were cultured and yielded coats of colony forming units. The isolates were confirmed by conventional multiplex PCR as previously described (Moravkova et al., 2008).

DISCUSSION AND CONCLUSIONS

Tuberculosis is a common cause of death in raptors. A number of reports describe the clinical dis-

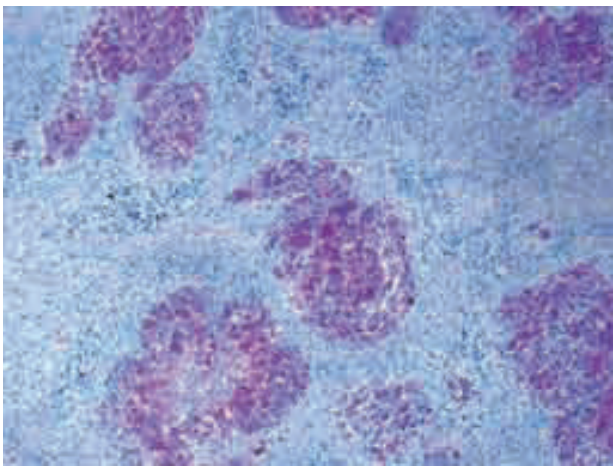

Figure 5. Spleen; vulture. **Massive presence of mycobacteria** in granulomas, Ziehl-Neelsen, 200×

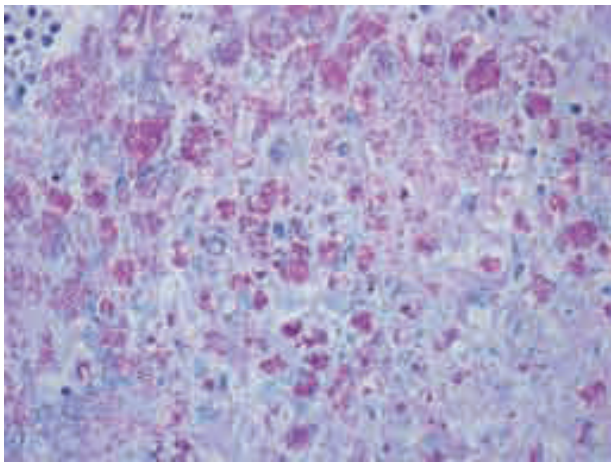

Figure 6. Liver; vulture. Macrophages within granuloma containing a huge number of rod-shaped mycobacteria, Ziehl-Neelsen, 1000×

ease and pathological lesions caused by *M. avium* (Lumeij et al., 1981; Schroder, 1981; Smit et al., 1987; Hoop et al., 1996; Hoenerhoff et al., 2004; Tell et al., 2004; Heatley et al., 2007; Millan et al., 2010). Interestingly, there is no previous report of avian tuberculosis in a vulture, despite records on morbidity and mortality of many different species of raptorial birds (Kaliner and Cooper, 1973; Lairmore et al., 1985; Morishita et al., 1998; Hoenerhoff et al., 2004; Tell et al., 2004; Heatley et al., 2007; Millan et al., 2010). The features of lesions caused by mycobacterial infections may vary according to the infected avian species. The majority of avian tuberculosis cases are of the disseminated visceral form, primarily involving the liver and spleen (Tell et al., 2001). Granuloma formation in the intestine and parenchymatous organs is the typical pathological feature of mycobacterial infection in raptorial birds (Hoop et al., 1996; Tell et al., 2001; Jones, 2006). In contrast, granulomas are generally absent in Columbiformes, Anseriformes and most Psittaciformes (Tell et al., 2001). In this instance, the affected organs were the liver and spleen and granulomas were not present in the intestine of the vulture. According to some authors, the natural resistance of birds of prey to mycobacterial infections is the reason for the low occurrence of tuberculosis in free-living raptorial birds. Therefore, tuberculosis is considered as a relatively rare disease (Piechocki, 1981; Witte et al., 2010). In comparison, other authors describe the occurrence of avian tuberculosis as a more frequent observation in captured raptors (Lumeij et al., 1981; Schroder, 1981; Tell et al., 2001; Jones, 2006; Witte et al., 2010).

The immune system of the infected bird may affect the feature of the lesions formed. The numbers of granulomas with necrotic centres increase over the numbers of granulomas with central diffuse histiocytic inflammation in stressed birds (Tell et al., 2001). In this case, the number of granulomas with central necrosis was significantly higher than the number of granulomas consisting of only epithelioid macrophages and lymphocytes and without the presence of central necrosis. Avian tuberculosis has great potential to be transmitted to other avian and mammalian species and also has zoonotic potential, especially in immunocompromised patients, such as those with *HIV*/*AIDS* (Tell et al., 2001).

Clinical diagnosis in raptors can be difficult due to non-specific clinical signs of the disease and treatment is often unsuccessful. Histopathological examination of affected organs, culture and quantitative Real-Time PCR examinations of specimens reliably confirm the diagnosis *post mortem*.

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