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Transmission of tuberculosis between men and pet bird – a case report

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Short title: Tuberculosis in a pet bird

Figure 4 to be in colour in hard copy version, free of charge.

Figures 1, 2, and 3 to be in black-and-white, each occupying a half-page or so

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Abstract

This report describes a case of *Mycobacterium tuberculosis* infection in an African Grey parrot (*Psittacus erithacus erithacus*) kept as a pet bird. Diagnosis was confirmed by microbiologic and pathologic results, and indicated a human-avian transmission. Clinical signs included sublingual nodules resulting in anorexia and signs of osteolysis in the long bones. Proliferation consisted of several nodules with small greenish-caseous foci in cross-section and revealed a severe granulomatous inflammation without intralesional acid-fast rods. *M. tuberculosis* was cultured from a pooled sample of sublingual and liver nodules and confirmed via PCR. Transmission between men and parrot was proved by spoligotyping pattern analysis. The absence of facial skin lesions and acid-fast rods within the tubercles is contrary to previous publications of tuberculosis in birds. This disease in a common pet bird species is of zoonotic importance, and those parrots with close contact to owners suffering from tuberculosis may serve as a potential reservoir for human infection.

Introduction

Mycobacterium tuberculosis primarily causes diseases in humans, and reports about infections with *M. tuberculosis* in birds are rare. Most of these reports describe the appearance in psittacine birds (principally the parrot family) beside one case in a canary (*Serinus canaria forma domestica*) (Hoop, 2002). In addition to five reports of infections in Amazon parrots [mealy amazon (*Amazona farinosa*) (Ackerman *et al.*, 1974), yellow-naped amazon (*Amazona orchocephala auropalliata*) (Woerpel *et al.*, 1983), red-lored amazon (*Amazona autumnalis*) (Brown, 1990), two blue-fronted amazons (*Amazona aestiva aestiva*) (Hoop, 2002; Peters *et al.*, 2007)] two reports in green-winged macaws (*Ara chloroptera*) (Washko *et al.*, 1998; Steinmetz *et al.*, 2006) exist. Cutaneous and subcutaneous nodules (Brown, 1990; Washko *et al.*, 1998, Hoop, 2002; Steinmetz *et al.*, 2006, Peters *et al.*, 2007), granulomas on the membrana nictitans (Ackerman *et al.*, 1974) and retrobulbar tissues (Woerpel *et al.*, 1983), in the infraorbital sinuses and nares (Brown, 1990) as well as in the oral cavity (Brown, 1990, Washko *et al.*, 1998; Peters *et al.*, 2007) were detected in these parrots. Adjacent to the mentioned localisations, systemic manifestation was documented regularly, macroscopically visible as granulomatous tubercles of lung and liver and confirmed by histopathological methods (Ackerman *et al.*, 1974; Brown, 1990; Washko *et al.*, 1998; Hoop, 2002; Steinmetz *et al.*, 2006; Peters *et al.*, 2007).

In most of the described cases there was no known contact of the birds with humans suffering from tuberculosis (Ackerman *et al.*, 1974; Woerpel *et al.*, 1983; Brown, 1990). Transmission between humans and parrots was suspected in two cases of green-winged macaws (Washko *et al.*, 1998; Steinmetz *et al.*, 2006) and could be recently confirmed by identical spoligotyping patterns of a blue-fronted amazon and its owner (Peters *et al.*, 2007). In those cases transmission was supposed due to close contact with owners suffering from

tuberculosis, who used to feed the birds with pre-chewed food. Because of facial skin lesions route of infection via aerosol was supposed (Peters *et al.*, 2007).

African grey parrots are the most frequently kept companion parrots due to their intelligent and playful character, and mostly have close emotional contact to the owners (Perry, 1994; Lennox and Harrison, 2006). Therefore, this reported case might have implications for public health, as this human-to-bird and, possibly, bird-to-human transmission of tuberculosis could represent a perpetual reservoir for human infection.

Materials and Methods

Case history. In December 2007, an adult African Grey Parrot was presented with a two-month history of sublingual nodules which resulted in disability of food intake. In 1996, the parrot was captured in Zaire, imported to Germany and then acquired by the owner. The measured body mass was 357 g and the bird showed a bad body condition. Two months before, the sublingual nodules were surgically removed by another veterinarian without further diagnostics.

At the time of presentation the parrot had a sublingual oedema and multiple white-yellow nodules with a dimension of up to 0.5 cm diameter (Figure 1). Furthermore skin lesions located at the right intertarsal and the right metacarpal joint were detectable. Leg lesions were yellow-coloured and had a knotty character with a dimension of up to 0.5 cm diameter. Some of these nodules showed erosions on the surface and a yellow cheesy content could be squeezed out. The wing lesion consisted of a complete crusty ulceration of the skin and subcutaneous swelling with a dimension of up to 1.0 cm diameter. Complete-body

radiographs in latero-lateral and ventro-dorsal positioning were performed. Radiographs revealed osteolytic changes on the right wing affecting ulna, radius and metatarsus (Figure 2).

The bird's owner had suffered from pulmonary tuberculosis and had completed the anti-tuberculosis treatment 2.5 years before presenting his parrot to a veterinarian. Because of this fact and the owner's habit of feeding the bird with pre-chewed food, tuberculosis was suspected in this parrot. In agreement with the owner the bird was euthanized within isofluran inhalant anaesthesia by intravenous injection of potassium chloride (2 mmol/kg body weight).

Post-mortem examination. Immediately after the death of the African Grey parrot, necropsy was performed according to standard procedures (Latimer and Rakich, 1994). Wet mounts of crop, large and small intestine were examined microscopically at 200x and 400x magnification for parasitological diagnostics. Swabs for bacteriological and mycological culture were taken from liver, lung, heart, kidneys and small intestine. Bacteriological cultures were performed on Columbia agar supplemented with defibrinated sheep blood and brilliant green agar (Oxoid, Wesel, Germany) and incubated at 38°C under aerobic conditions for 24 h. Mycological cultures were performed on Sabouraud's dextrose agar (Oxoid) and incubated at 38°C for 72 h. For detection of *Salmonella* species, samples of liver and the intestinal contents were inoculated into selenite lactose medium (Oxoid) and the cultures incubated at 38°C for 48 h. Samples were subcultured on Xylose Lactose Tergitol™ 4 agar (Oxoid) at 38°C for 24 h.

Stained impression smears (DiffQuik®; Dade Behring, Marburg, Germany) prepared from liver, spleen, lung, kidney, sublingual nodules and small intestines were examined microscopically at 1000x magnification. Additionally impression smears of the mentioned organs were stained by acid-fast (Ziehl Neelsen stain) techniques and examined microscopically at 1000x magnification.

Sections of skin, spleen, liver, lung, kidney, brain, heart, muscles, thyroids, proventriculus, gizzard, pancreas, small and large intestine were fixed in 10% neutral-buffered formalin for at least 24 h. Sections of bones were decalcified (Ossa fixona[®]; Diagonal, Münster, Germany) for at least 48 h. Formalin-fixed samples were dehydrated, routinely embedded in paraffin wax and sectioned at 4 µm. All sections were stained with haematoxylin and eosin, periodic acid–Schiff (PAS) and acid-fast (Ziehl Neelsen stain) techniques. Additionally, liver, kidney and spleen samples were stained with Prussian blue iron stain and Turnbull blue iron stain.

Microbiological methods. A pooled tissue sample from sublingual-, bone- and liver-tubercles was sent to the National Reference Centre for Mycobacteria (Borstel) for examination of the presence of mycobacteria. The sample was processed according to national guidelines (DIN). Microscopic slides were prepared directly from the tissue samples which have been minced in a tissue grinder before the decontamination procedure and stained according to the Ziehl-Neelsen technique. Each sample was decontaminated, homogenized and concentrated using the N-acetyl-L-Cystein-sodium hydroxide method (DIN).

The decontaminated sample was inoculated onto two sets of solid slants (Löwenstein-Jensen, Stonebrink, Middlebrook 7H10), into a BACTEC MGIT 960 tube, and into a BACTEC 460TB vial (Becton Dickinson and Company, Cockeysville, USA). One set of solid slants and the BACTEC MGIT 960 tube were incubated at 37°C, the second set of solid slants and the BACTEC 460TB vial were incubated at 31°C. Solid slants were incubated for eight weeks and inspected for growth once weekly. MGIT tubes were incubated in and automatically read by the BACTEC MGIT instrument. BACTEC 460TB vial were incubated for 6 weeks and read according to the manufacturer's protocol.

Positive cultures were identified using the Genotype Mycobacterium CM and the Genotype MTBC assays (Hain Lifescience, Nehren, Germany).

For the comparison of strain identity the spoligotyping method was performed according to standard procedures (Kamerbeek *et al.*, 1997). This technique is based on the detection of various non-repetitive spacer sequences located between small repetitive units in the DR-locus.

The *M. tuberculosis* strain of the bird's owner, which was isolated in the 2005, was kindly provided by a local laboratory.

Results

Post mortem findings. During necropsy, multiple sublingual nodules with a yellow friable content could be found into the oral cavity down to the cranial part of the neck (Figure 1). The liver, showing a red-green mottled colour, demonstrated bifocal nodules of yellow colour, solid consistence and a dimension of 0.5 cm diameter (Figure 3). No abnormal findings were pathologic-anatomically detected in other organs. Bacteria or fungi could not be cultivated and parasites were not detectable.

Cytological and patho-histological findings. In the cytological imprint of the liver, inflammatory cells were detectable at a high number consisting of heterophile granulocytes, lymphocytes and monocytes. Liver cell nuclei exhibited anisocaryosis. Kidney imprint revealed lots of bluish inclusions. Acid fast staining of all organ imprints was without further findings and especially negative for acid fast rods.

Pathohistologically granulomatous inflammatory reaction could be shown in bones, skin, sublingual mucosa and liver. Granulomas appeared as typical tubercles consisting of central

necroses which were surrounded by polynucleated giant cells, epithelioid cells and peripheral fibroplasia (Figure 4). Acid-fast bacteria were not detectable in any granuloma (Figure 4).

Multifocal iron phagocytosis was detectable in spleen and also discursively in hepatocytes and renal tubular epithelium. Other organs appeared with no abnormal histopathological findings.

Microbiological results. Ziehl-Neelsen staining for the presence of acid-fast bacteria in the tissue was negative. Liquid MGIT medium of the tissue samples became positive after 13 days of incubation. The presence of *M. tuberculosis* complex was assessed with the Genotype Mycobacterium CM assay. The final identification of *M. tuberculosis* was obtained with the Genotype MTBC assay. Within 27 days, all liquid and solid cultures became positive with *M. tuberculosis*.

The *M. tuberculosis* isolates of the bird as well as of the owner were analysed with the spoligotyping method. Both strains showed the same pattern confirming transmission of the strain between man and animal (Figure 5).

Discussion

M. tuberculosis was isolated from the tissue of the bird and identified by molecular methods. Identical spoligotyping patterns of the avian and human *M. tuberculosis* isolates indicate a zoonotic link between men and pet bird. As parrots do not represent primary target hosts for *M. tuberculosis*, it is most likely that the infection route in this case was from human to animal. With regard to anamnestic and pathomorphological findings, the reported case reveals remarkable parallels to disseminated *M. tuberculosis* infection in a green-winged macaw

(Washko *et al.*, 1998) and to the infection of a blue-fronted amazon (Peters *et al.*, 2007). In both cases as well as the present case, owners with confirmed tuberculosis used to feed the parrots from their mouths. Subsequent formation of sublingual tubercles was suspicious of direct transmission of *M. tuberculosis* from mouth or sputum from men to the beak cavity of the parrots. The long incubation period of 2.5 years in the present case is in accordance with other cases known from literature, in which incubation periods were estimated between 0.5 and 4 years, respectively (Washko *et al.*, 1998; Steinmetz *et al.*, 2006; Peters *et al.*, 2007). In a recently published experimental infection study, a long incubation period for *M. tuberculosis* infection could also be confirmed in budgerigars (Ledwon *et al.*, 2008). Contrary to the clinical signs described above for the blue-fronted amazon and the green-winged macaw, facial lesion were absent in the African Grey parrot. Focal skin lesion on the wing and the leg nevertheless could be seen, so that a transmission via aerosols could not be ruled out.

However, any parrot with ocular, sinus, oral, cutaneous nodular lesions (Ackerman *et al.*, 1974; Brown, 1990; Washko *et al.*, 1998; Hoop, 2002; Steinmetz *et al.*, 2006; Peters *et al.*, 2007) and/or osteolytic bones (Steinmetz *et al.*, 2006) on radiographs, and a history of tuberculosis of the owners (Washko *et al.*, 1998; Peters *et al.*, 2007) should be considered suspicious for *M. tuberculosis* infection. The absence of facial skin lesions, which are frequently reported, should not rule out tuberculosis. This case also reveals that acid-fast staining is not sufficient for exclusion of a mycobacterial infection. Particularly in cases of infection with *M. tuberculosis*, which is typically found in relatively small numbers in infected tissue of birds, cultures might be positive even if acid-fast staining is negative. In those cases, the infected pet has to be considered as a health risk for its owners (Brown, 1990; Washko *et al.*, 1998; Hoop, 2002; Steinmetz *et al.*, 2006; Peters *et al.*, 2007). In order to rule

out contact birds as a potential source for re-infection, long-term observation of these animals is recommended.

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Legends to Figures.

Figure 1. *Multiple white-yellow sublingual nodules with a dimension of up to 0.5 cm diameter and a yellow friable content, African Grey parrot, post mortem.*

Figure 2. *Osteolytic changes on the right wing affecting ulna, radius and metatarsus, African Grey parrot, radiograph ventrodorsal view.*

Figure 3. *Liver showing a red-green mottled colour and bifocal nodules of yellow colour with solid consistence and a dimension of 0.5 cm diameter, African Grey parrot, situs post mortem.*

Figure 4. *Light micrograph of granulomas consisting of central necroses which were surrounded by polynucleated giant cells, epithelioid cells and peripheral fibroplasia. (A) Sublingual granuloma. HE stain: 200x magnification. (B) Sublingual granuloma. HE stain: 200x magnification. (C) Granuloma in the liver. HE stain: 400x magnification. (D) Acid-fast bacteria were not detectable in any granuloma. Sublingual granuloma, Ziehl-Neelsen stain: 200x magnification.*

Figure 5. *Spoligotyping patterns of the M. tuberculosis strains of the parrot and owner of the parrot.*



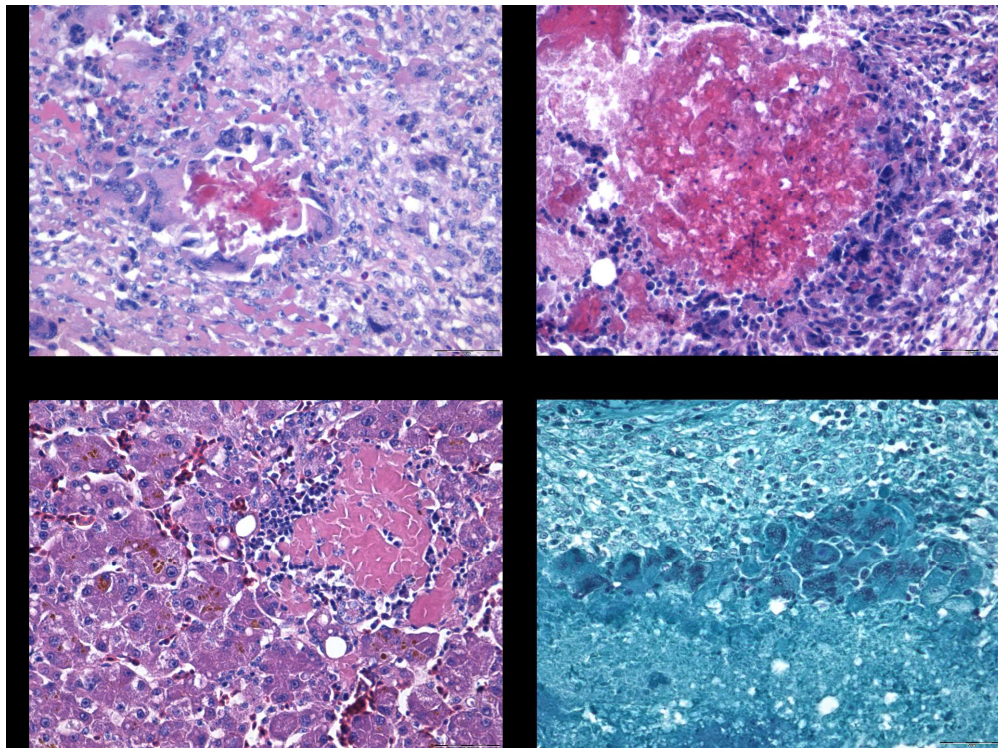
Multiple white-yellow sublingual nodules with a dimension of up to 0.5 cm diameter and a yellow friable content, African Grey parrot, post mortem.
541x722mm (72 x 72 DPI)



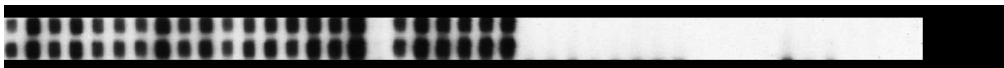
Osteolytic changes on the right wing affecting ulna, radius and metatarsus, African Grey parrot, radiograph ventrodorsal view.
20218x32918mm (1 x 1 DPI)



Liver showing a red-green mottled colour and bifocal nodules of yellow colour with solid consistence and a dimension of 0.5 cm diameter, African Grey parrot, situs post mortem.
722x541mm (72 x 72 DPI)



Light micrograph of granulomas consisting of central necroses which were surrounded by polynucleated giant cells, epithelioid cells and peripheral fibroplasia. (A) Sublingual granuloma. HE stain: 200x magnification. (B) Sublingual granuloma. HE stain: 200x magnification. (C) Granuloma in the liver. HE stain: 400x magnification. (D) Acid-fast bacteria were not detectable in any granuloma. Sublingual granuloma, Ziehl-Neelsen stain: 200x magnification. 264x196mm (200 x 200 DPI)



Spoligotyping patterns of the *M. tuberculosis* strains of the parrot and owner of the parrot.
228x14mm (150 x 150 DPI)

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