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M. J. L. Kik, D. J. Houwers and A. Dinkla

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# Short Communications

## *Mycobacterium intermedium* granulomatous pneumonia in a green oropendola (*Psarocolius viridis*)

M. J. L. Kik, D. J. Houwers, A. Dinkla

MYCOBACTERIAL infections in captive exotic birds are mainly caused by *Mycobacterium avium* and *Mycobacterium genavensis* (Tell and others 2001). *M. avium* causes infections in the widest range of vertebrate hosts, and is the most widely distributed mycobacterial species. Cases of clinical disease caused by other mycobacteria are seldom described in birds. Most non-tuberculous mycobacteria (NMT), which include the mycobacteria that are not members of the *Mycobacterium tuberculosis* complex, are widespread throughout the environment, including water and soil (Covert and others 1999, Cromie and others 2000). NMT are opportunistic organisms and not obligate pathogens. NMT have emerged as a major cause of opportunistic infections in immunocompromised hosts. From the 1950s onwards, various forms of lung disease associated with NMT have been identified, and numerous reports on recently detected and potentially pathogenic species have been published (Schönfeld 2006). *Mycobacterium intermedium*, a slow-growing mycobacterial species was first reported in 1993 after being isolated from the sputum of a human patient with pulmonary disease (Meier and others 1993). Only a few reports of *M. intermedium* infections in human patients, all non-immunocompromised people, have been published. One describes a non-immunosuppressed patient with chronic *M. intermedium* granulomatous dermatitis acquired after exposure to the bacterium in a hot tub (Edson and others 2006); in another case, the patient suffered from pulmonary *M. intermedium* disease (Ito and others 2005). This short communication describes a case of pulmonary disease associated with *M. intermedium* in a green oropendola (*Psarocolius viridis*).

A four-year old, captive-born, female green oropendola kept as part of a zoological collection was presented to the zoo veterinarian. The bird showed dyspnoea. It was treated with enrofloxacin (Baytril

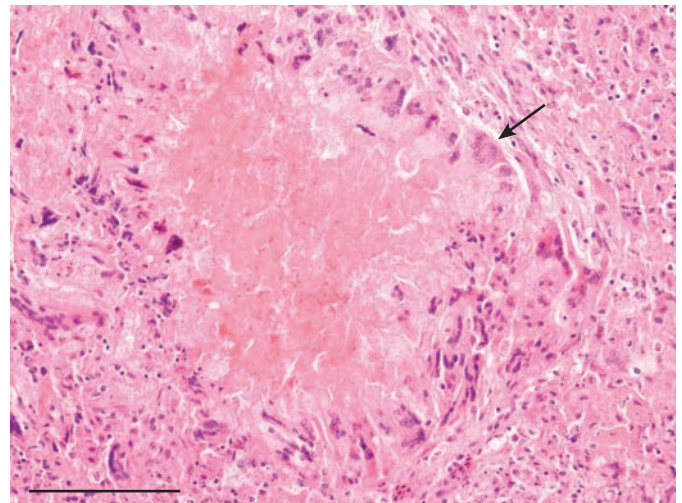


FIG 1: Pulmonary lesion, consisting of a granuloma with multinucleated giant cells (arrow), in a green oropendola (*Psarocolius viridis*). Haematoxylin and eosin. Bar=100  $\mu$ m

2.5 per cent; Bayer), 0.1 ml administered orally once a day for 14 days, and toltrazuril (Baycox 2.5 per cent; Bayer), 25 mg/l drinking water, for two days. Blood chemistry revealed a lowered albumin fraction and elevated  $\alpha$ ,  $\beta$  and  $\gamma$  globulins and an albumin:globulin ratio of 0.31, indicating chronic disease. Treatment with itraconazole (Trisporal; Janssen-Cilag), 0.3 ml administered in the bird's feed once a day, was started. Despite the treatment, the bird's clinical condition deteriorated; because of this, and the severe dyspnoea, it was euthanased.

At postmortem examination, based on the fat stores and muscle development the bird was judged to be in a poor nutritional state. The main pathological changes were located in the lungs and air sacs. Multifocal granulomatous lesions, ranging in size from 4 mm to 1 cm, were randomly distributed throughout the lungs and the air sacs. The other organs appeared unaffected.

After postmortem examination, only the lungs of the bird were submitted for further pathological examination. Impression smears were made from the lung tissue and stained with Hemacolor (Merck) and Ziehl-Neelsen (ZN) stain. Tissue samples from the lung were collected for histology and fixed in 4 per cent phosphate-buffered formalin, embedded in paraffin, sectioned at 4  $\mu$ m and stained with haematoxylin and eosin and ZN. Duplicate tissue samples were stored at  $-80^{\circ}\text{C}$  before bacteriological culture.

Histologically, the pathological changes in lungs were characterised by multifocal necrosis surrounded by degenerate heterophilic granulocytes, multinucleate giant cells and epitheloid macrophages (Fig 1). More diffusely scattered fibrin deposits, multinucleate giant cells, plasma cells, lymphocytes and many heterophils were present in the adjacent lung tissue. ZN stain revealed many short, rod-shaped acid-fast bacilli in the areas of necrosis and within macrophages and multinucleated giant cells (Fig 2).

Tissue samples were decontaminated according to the method described by Beerwerth (1967) and cultivated on modified Löwenstein-Jensen medium supplemented with 4 mg/ml pyruvate (Jørgensen 1982). The first colonies were isolated after three weeks of incubation. After six weeks of incubation all slants were fully overgrown. A single mycobacterial species was isolated.

DNA extracts were made from pure cultures of mycobacteria by boiling for 10 minutes. A 500 bp 16S rDNA fragment was amplified by PCR using the MicroSeq 500 system 16S rDNA bacterial sequencing kit (Applied Biosystems) according to the manufacturer's instructions. The PCR products were then sequenced using an ABI 3130 Genetic Analyzer (Applied Biosystems) according to the manufac-

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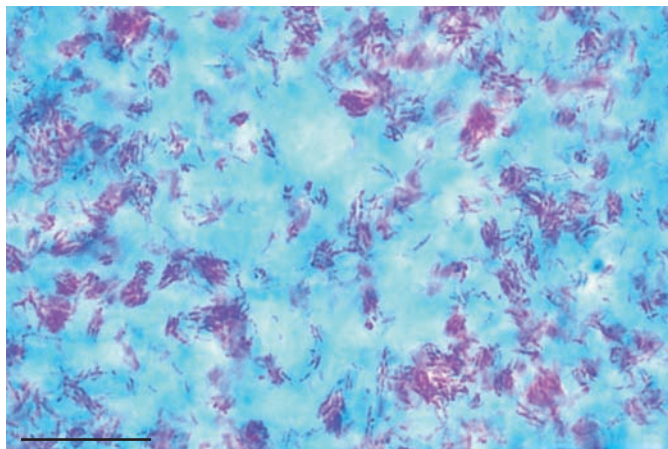


FIG 2: Acid-fast rod-shaped bacteria within a necrotic granuloma in the lung of a green oropendola (*Psarocolius viridis*). Ziehl-Neelsen stain. Bar=20  $\mu$ m

turer's instructions. The obtained 16S rDNA sequence was compared with the NCBI database ([www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/)) using BLASTN. On the basis of this comparison, the mycobacterial species was identified as *M intermedium*.

NMT can cause opportunistic infections in (usually) immunocompromised human beings and animals and are widespread throughout the environment. In the present case, the aetiological agent of the granulomatous pneumonia and airsacculitis observed in the oropen-

dola was *M intermedium*. The few reports of human *M intermedium* infections were in non-immunosuppressed individuals. The extensive granulomatous lesions in the lungs and air sacs of the oropendola suggest that it had acquired *M intermedium* infection through inhalation. Exposure to the agent from soil or water in the bird's enclosure is the most probable source. *M intermedium* should be added to the list of agents causing mycobacteriosis in birds.

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