J. Comp. Path. 2016, Vol. ■, 1–4

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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Non-tuberculous Mycobacteriosis with T-cell Lymphoma in a Red Panda (*Ailurus fulgens*)

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Summary

A 9-year-old male red panda (*Ailurus fulgens*) became emaciated and died. Necropsy examination revealed systemic lymphadenomegaly. The liver, lungs and left kidney contained multifocal yellow nodules. Microscopical examination revealed granulomatous inflammation in the liver, lungs, kidney, spleen and lymph nodes, with numerous acid-fast bacilli. Sequencing of genetic material isolated from the tissues classified the pathogen as *Mycobacterium gastri*. Lymphoma was found in the liver, lungs, kidney and lymph nodes. The neoplastic cells were strongly labelled for expression of CD3, Ki67 and proliferating cell nuclear antigen by immunohistochemistry. This is the first report of *M. gastri* infection with T-cell lymphoma in a red panda.

Keywords: *Mycobacterium gastri*; non-tuberculous mycobacteriosis; red panda; T-cell lymphoma

Nontuberculous mycobacteria (NTM) are ubiquitous in the environment and are potential pathogens for man and animals. More than 140 mycobacterial species have been reported in people and NTM are common pathogens in immunodeficient patients (Tortoli *et al.*, 2000). In man, the main pathogens causing non-tuberculous mycobacteriosis are *Mycobacterium avium–intracellulare* complex (MAC) (Tsukamura *et al.*, 1988; Biet *et al.*, 2005), which induce respiratory disease. Non-tuberculous mycobacteriosis is mainly caused by MAC in many animals including, deer, horses, pigs and birds. In pigs, *M. avium* usually causes granulomatous lesions in the mesenteric lymph nodes, liver, lungs and spleen (Biet *et al.*, 2005).

NTM can affect immunosuppressed human patients with lymphoma and acquired immunodeficiency syndrome (AIDS) (Jacobson *et al.*, 1991; Chin *et al.*, 1994). Mycobacterial infection has also been reported in an immunosuppressed ferret (Saunders and Thomosen, 2006). Chronic inflammation can also cause tumour formation; for example, macrophages stimulated by *Helicobacter pylori* release a proliferation-inducing ligand, which belongs to the tumour necrosis factor family. B lymphocytes are stimulated to maturity by this cytokine and mucosa-associated lymphoid tissue (MALT) lymphoma develops (Westbrook *et al.*, 2010; Sebastian and Hana, 2014).

Mycobacterium gastri is a NTM first detected by human gastric lavage (Wayne, 1966) and is a known factor in the development of peritonitis and septic metacarpophalangeal arthritis, seminal vesiculitis and lymphadenitis in man (Linton *et al.*, 1986; Indudhara *et al.*, 1991; Perandones *et al.*, 1991; Velayati *et al.*, 2005). However, there are no reports of *M. gastri* infection in animals. We present a case of non-tuberculous mycobacteriosis due to *M. gastri* with T-cell lymphoma formation in a red panda (*Ailurus fulgens*). To our knowledge, this is the first report

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0021-9975/$ - see front matter

http://dx.doi.org/10.1016/j.jcpa.2016.06.001

Please cite this article in press as: Fuke N, et al., Non-tuberculous Mycobacteriosis with T-cell Lymphoma in a Red Panda (*Ailurus fulgens*), Journal of Comparative Pathology (2016), http://dx.doi.org/10.1016/j.jcpa.2016.06.001

of *M. gastri* infection with T-cell lymphoma in this species.

A 9-year-old neutered male red panda in a zoological garden in Japan was observed to have a cataract in the right eye and dyskeratosis throughout the body after transportation from another zoo in winter. Two months later, lymph node biopsy was performed because of lymphadenomegaly and microscopical examination revealed lymphadenitis. Neoplastic changes were not observed at that time. Four months later, the animal showed emaciation and died. A necropsy examination was performed at the Department of Veterinary Pathology, University of Miyazaki. The other red pandas in the group appeared clinically normal.

At necropsy examination, the lymph nodes, especially the right axillary, inguinal, mandibular, popliteal and mesenteric lymph nodes were enlarged. Multiple, diffuse, yellow foci, 2–5 cm in diameter, were observed in all lung and liver lobes. The cut surface of these foci and of the lymph nodes was white and roughened. There was serous atrophy of the perirenal fat. Many white spots (3–4 mm diameter) were seen in the cortex of the left kidney. The abdominal cavity contained yellow fluid.

Samples of the lungs, liver, heart, intestine, spleen, kidneys and lymph nodes were collected, fixed in 10% neutral buffered formalin, routinely processed and embedded in paraffin wax. Sections (2 μm) were stained with haematoxylin and eosin (HE) and Ziehl–Neelsen stain. Immunohistochemistry (IHC) was performed with primary reagents specific for CD3, CD20, proliferating cell nuclear antigen (PCNA) and Ki67 (Dako, Glostrup, Denmark).

Parts of the excised liver lesion were submitted for bacterial culture on 2% Ogawa egg yolk medium at the Department of Veterinary Microbiology, University of Miyazaki. Polymerase chain reaction (PCR), using primers for the *Mycobacterium*16S rRNA gene, was performed on colonies from the culture medium and sequencing was conducted (Shin *et al.*, 2009). A DNA–DNA hybridization method for 22 *Mycobacterium* spp. was conducted using DDH–mycobacteria Kyokuto (Kyokuto, Tokyo, Japan), according to methods described previously (Kusunoki *et al.*, 1991). Conventional biochemical tests, photochromogenicity and nitrate reduction, were conducted to distinguish between *M. gastri* and *Mycobacterium kansasii* (Tsukamura, 1967; Tsukamura, 1973).

Microscopical examination of the liver (Fig. 1), lungs, left kidney and the lymph nodes revealed a mixture of granulomatous inflammation and neoplastic lymphocytes. The liver parenchyma showed diffuse granulomatous inflammation with central necrosis surrounded by macrophages, multinucleated giant cells, neutrophils and lymphocytes (Fig. 2). There was abundant fibrosis and mineralization around these areas of granulomatous inflammation. Similar findings were observed in the lungs, left kidney, spleen and lymph nodes. Ziehl–Neelsen staining revealed numerous acid-fast bacilli in the cytoplasm of macrophages and extracellular areas in the liver, lungs, left kidney, spleen and lymph nodes (Fig. 3). Normal lymph node structure was replaced by granulomatous inflammation and necrosis.

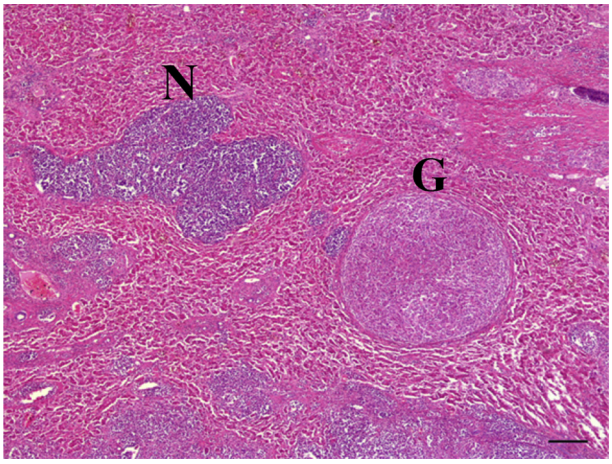

Fig. 1. A mixture of granulomatous inflammation (G) and foci of neoplastic lymphocytes (N) in the liver. HE. Bar, 200 μm.

Foci of neoplastic cells were found around the portal vein and invading lymphatic vessels in the liver (Fig. 4A). Neoplastic cells were round and pleomorphic with large, irregular nuclei that contained a single prominent nucleolus. The mitotic rate was 2 (average number of mitoses over ten ×400 fields)

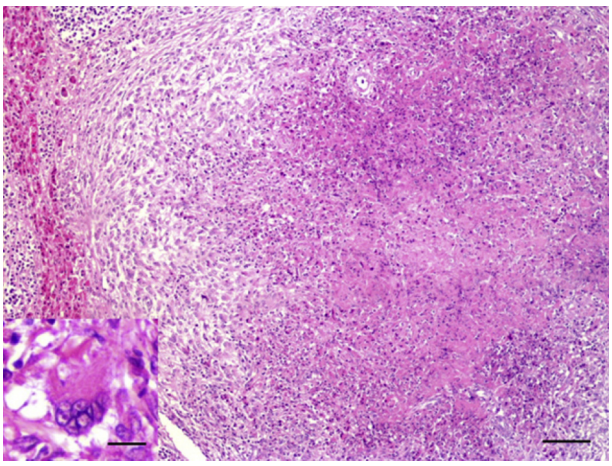

Fig. 2. Granulomatous inflammation consisting of necrosis, macrophages, multinucleated giant cells and neutrophils in the liver. HE. Bar, 100 μm. Inset: multinucleated giant cells. HE. Bar, 20 μm.

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