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MYCOBACTERIUM INTRACELLULARE INFECTION CAUSING A RETROPERITONEAL MASS IN A BINTURONG (*ARCTICTIS BINTURONG*)

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Abstract: A 19-yr-old castrated male binturong (*Arctictis binturong*) with a history of recurrent pyogranulomatous panniculitis, lymphangitis, and dermatitis was presented for evaluation of hyporexia and tenesmus. A large caudal abdominal mass was palpated on physical examination. On ultrasound, the mass encircled and obstructed the left ureter, resulting in hydroureter and hydronephrosis. The animal was euthanized, and necropsy revealed a large retroperitoneal pyogranuloma with acid-fast organisms identified in both the mass and the perineal skin. The acid-fast organisms within the retroperitoneal mass were identified as *Mycobacterium intracellulare* by PCR. This case represents an unusual presentation of *M. intracellulare* in a novel species.

Key words: *Arctictis binturong*, binturong, mycobacteriosis, *Mycobacterium intracellulare*, pyogranuloma.

BRIEF COMMUNICATION

An 18-yr-old castrated male binturong (*Arctictis binturong*) was presented for a 4-yr history of recurrent inguinal dermatitis and edema. Previous serial skin scrapes and dermatophyte cultures revealed no significant findings, and empirical treatment with multiple combinations of steroids and antibiotics failed to significantly improve clinical signs. The animal also had a history of persistent priapism and unilateral testicular enlargement managed with castration 3 yr prior to presentation. Histopathology of the enlarged testis was reportedly unremarkable.

Physical examination under general anesthesia revealed persistent priapism and cutaneous abnormalities including inguinal alopecia with moderate to severe erythema and hyperpigmentation, subcutaneous nodules, edema, and skin thickening (Fig. 1). Hematology and biochemistry evaluation showed a thrombocytosis ($754 \times 10^3/\mu l$, reference interval [RI] $107–614 \times 10^3/\mu l$) and mild leukocytosis ($25.74 \times 10^3/\mu l$, RI $5.03–21.89 \times 10^3/\mu l$) characterized by a neutrophilia ($21.622 \times 10^3/\mu l$, RI $2.89–15.47 \times 10^3/\mu l$) consistent with a chronic inflammatory process.[7] Mild hypoalbuminemia was also present (2.4 g/dl, RI 2.9–5.2 g/dl).[7] Skin biopsies collected from the inguinal region revealed marked nodular pyogranulomatous panniculitis, lymphangitis, and dermatitis with marked lymphatic dilation and dermal edema. Gram, Giemsa, Fite–Faraco acid-fast, Warthin–Starry, and Grocott–Gomori methenamine silver (GMS) stains failed to reveal infectious organisms within the biopsy sections examined.

Treatment was initiated with marbofloxacin (Zeniquin, Zoetis Inc., Kalamazoo, Michigan 49007, USA: 5 mg/kg p.o. q. 24 hr × 30 days) and azithromycin (Sandoz Pharmaceuticals, Princeton, New Jersey 08540, USA; 10 mg/kg p.o. q. 24 hr × 30 days) and significant improvement was noted. However, clinical signs recurred when treatment was discontinued, so the antibiotic course was repeated. As before, the clinical signs improved with treatment and worsened when antibiotics were discontinued. Thus, long-term antibiotic therapy was initiated. After 12 mo of concurrent marbofloxacin and azithromycin therapy, the binturong's clinical signs were mostly controlled and it was weaned onto azithromycin alone (10 mg/kg p.o. q. 48 hr).

Eighteen months after the initial exam, the binturong was presented for evaluation of tenesmus and hyporexia of 10 days' duration. Anesthetized physical examination revealed a large (14 × 7 × 6 cm), firm, lobulated mass in the caudal abdomen, persistent priapism, an edematous left

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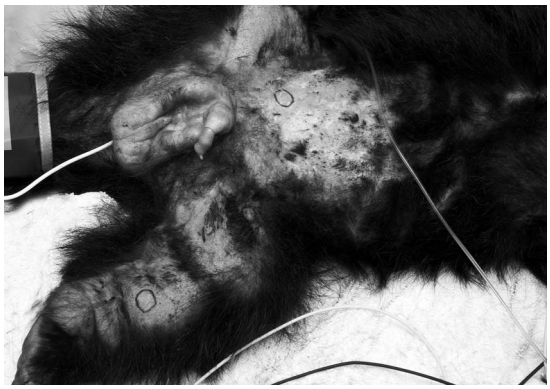

Figure 1. Appearance of the ventral abdomen, inguinal skin, and medial pelvic limbs in an 18-yr-old castrated male *A. binturong* at initial presentation. The skin of the ventral abdomen and of the inguinal and medial thigh regions exhibits moderate to severe erythema and hyperpigmentation. Palpation revealed multifocal subcutaneous nodules coalescing into a more diffuse thickening of the skin. No ulcerations or draining tracts were identified at any point during the examination. The left tarsus is edematous. Black circles represent proposed skin biopsy sites.

tarsus, and inguinal alopecia with associated thickened skin.

A complete blood count revealed progressive leukocytosis ($34.31 \times 10^3/\mu l$, RI $5.03–21.89 \times 10^3/\mu l$) characterized by a moderate–marked neutrophilia ($28.477 \times 10^3/\mu l$, RI $2.89–15.47 \times 10^3/\mu l$), mild monocytosis ($1.716 \times 10^3/\mu l$, RI $0.57–1.58 \times 10^3/\mu l$), and moderate thrombocytosis ($728 \times 10^3/\mu l$, RI $107–614 \times 10^3/\mu l$).[7] A plasma biochemistry profile showed a mild hyperkalemia (7.7 mM/L, RI 3.4–7 mM/L), mild hypocalcemia (7.1 mg/dl, RI 8.1–11.3 mg/dl), mild hypoalbuminemia (2.8 g/dl, RI 2.9–5.2 g/dl), and moderate hyperamylasemia (4,714 IU/L, RI 0–3,325 IU/L).[7]

Thoracic radiographs demonstrated a moderate diffuse bronchial pattern, but no evidence of metastatic disease. An abdominal ultrasound revealed a heterogenous, highly vascular mass encircling the left ureter and extending caudally to the urinary bladder. The left ureter was dilated (0.6–1.0 cm) proximal to the mass. The left renal pelvis (0.26 cm) and renal diverticula were also dilated. The kidneys were of similar size (left 6.9 cm, right 7.0 cm), and both contained small cortical cysts, hyperechoic cortices, and poor corticomedullary definition. Adjacent to the aorta, within the midmesentery, multiple varying-sized (1.0–1.4 cm) nodules were present, some of which had internal cystic regions. These nodules were most likely periaortic lymph nodes.

Sonographic findings were consistent with a left ureteral–periureteral mass with secondary ureteral obstruction and resultant pyelectasia–hydronephrosis. Tissue of origin differentials for the left ureteral mass included the ureter, peritoneum–mesentery, hypaxial muscle, or possibly a cryptorchid testicle. Neoplasia was the primary differential diagnosis, with a granuloma also considered. The remainder of the sonographic findings were consistent with bilateral nonspecific nephropathy, cystic renal cortical degeneration, and reactive or metastatic periaortic lymphadenopathy.

Exploratory laparotomy for surgical excision of the mass was discussed with the owners. However, because of guarded prognosis, the binturong was euthanized.

Gross necropsy revealed a $14 \times 6.5 \times 6$-cm firm, tan, multilobulated mass within the left retroperitoneal space extending 1 cm cranial to the left kidney and caudally into the pelvic canal. The mass was firmly adhered to the dorsal body wall, surrounded the left ureter, and multifocally invaded the left renal cortex (Fig. 2). The left renal pelvis and proximal ureter were dilated and multifocal cortical cysts were identified in both kidneys.

On histopathologic evaluation the retroperitoneal mass was composed of large, discrete islands of epithelioid macrophages, occasional multinucleated giant cells with up to five nuclei, degenerative neutrophils, and fewer small lymphocytes (Fig. 3). The islands of inflammatory cells were surrounded by thick, concentric bands of mature collagen. Inflammatory cells multifocally infiltrated and effaced the superficial left renal cortex. Within the perineal skin, the subcutis and dermis were multifocally markedly expanded by coalescing tracts of pyogranulomatous inflammation as described for the retroperitoneal mass (Fig. 3). Autolytic changes were observed within the kidney, small intestine, mesenteric lymph nodes, thyroid gland, and pancreas.

Fite–Faraco acid-fast, Ziehl–Neelsen acid-fast, GMS, and Giemsa stains were applied to sections of the retroperitoneal mass and perineal skin. Multiple clusters of Fite–Faraco- and GMS-positive bacilli were found within dense pyogranulomatous infiltrates of both tissues (Fig. 3). This staining pattern was most compatible with either *Nocardia* sp. or atypical *Mycobacterium* sp.

Mycobacterial DNA was PCR amplified from frozen sections of the retroperitoneal mass using universal *Mycobacterium* sp. primers as previously described.[12] The amplicon was sequenced in both

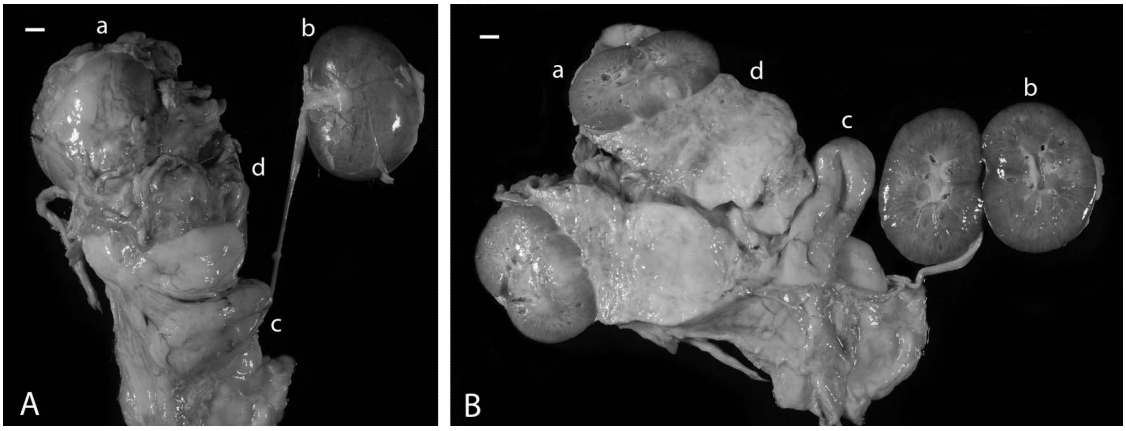

Figure 2. Gross appearance of the urogenital tract in a 19-yr-old castrated male *A. binturong* prior to (**A**) and after (**B**) sectioning of the kidneys and retroperitoneal mass: left kidney (a), right kidney (b), urinary bladder (c), and retroperitoneal mass (d). The white line is 1 cm. Multifocal renal cortical cysts are apparent.

directions and *Mycobacterium intracellulare* was confirmed by comparison to GenBank sequences (100% sequence identity with GenBank accession no. CP009499). A mycobacterial culture was attempted from frozen tissue sections, but was unsuccessful.

M. intracellulare is a ubiquitous environmental bacterium. It is a member of the *Mycobacterium avium* complex, and is considered an opportunistic pathogen.[14,15] Infections with *M. intracellulare* are well documented in humans, pigs, and several other domestic and exotic species.[1,2,5,6,9–11,13–15] *M. intracellulare* enters a host via ingestion, inhalation, or inoculation of wounds. The exact source of infection is often difficult to determine because of the long disease course. Infections have been documented in both immunocompetent and immunocompromised people.[1,5]

M. intracellulare lesion type and distribution is species dependent. In humans, *M. intracellulare* is typically associated with pulmonary infections, though cutaneous lesions with or without systemic dissemination have also been described.[5] Human cutaneous *M. intracellulare* lesions consist of papules, subcutaneous nodules, abscesses, ulcers, panniculitis, draining sinuses, and/or granulomatous plaques.[1,5] In swine, *M. intracellulare* infection typically causes clinically silent lymphadenitis.[14,15] However, disseminated *M. intracellulare* in pigs can cause expansive "neoplastic-like" lesions in addition to the more typical nodular pyogranulomas. These neoplastic-like lesions are large, fibrous, and nonencapsulated and lack a definitive caseous or purulent focus, yielding a gross appearance consistent with a neoplasm.[14] *M. intracellulare* lesions described in other species have included oral, laryngeal, and tracheal plaques in little blue penguins (*Eudyptula minor*), disseminated infections in opossums (*Didelphis virginiana*), and osteomyelitis with or without dissemination in multiple wallaby species.[2,9–11,13–15]

The binturong in this report developed a large retroperitoneal mass associated with *M. intracellulare* infection. The gross and sonographic appearance of the mass was consistent with a neoplastic process and histopathologic evaluation followed by molecular characterization was necessary to determine the nature of the lesion. The formation of abnormally large, neoplastic-like granulomatous lesions may be possible in all animals with *M. intracellulare* infection, or may be due to the unique host response of swine and binturongs to this infection. This binturong also had inguinal and perineal alopecia, erythema, and subcutaneous nodules histologically characterized by pyogranulomatous infiltrates containing Fite–Faraco– and GMS-positive bacilli. These findings most likely represent concurrent *M. intracellulare* infection within these tissues, though molecular confirmation was not obtained.

The binturong was maintained outdoors with many opportunities for environmental exposure to *M. intracellulare*. Binturongs are largely arboreal, but use the ground to move between trees.[16] Behavioral abnormalities increasing this animal's contact with potentially contaminated soil or water were not documented. Although this binturong's immune status was not fully investigated, neoplasia and canine distemper virus infection, two potentially immunosuppressive diseases pre-

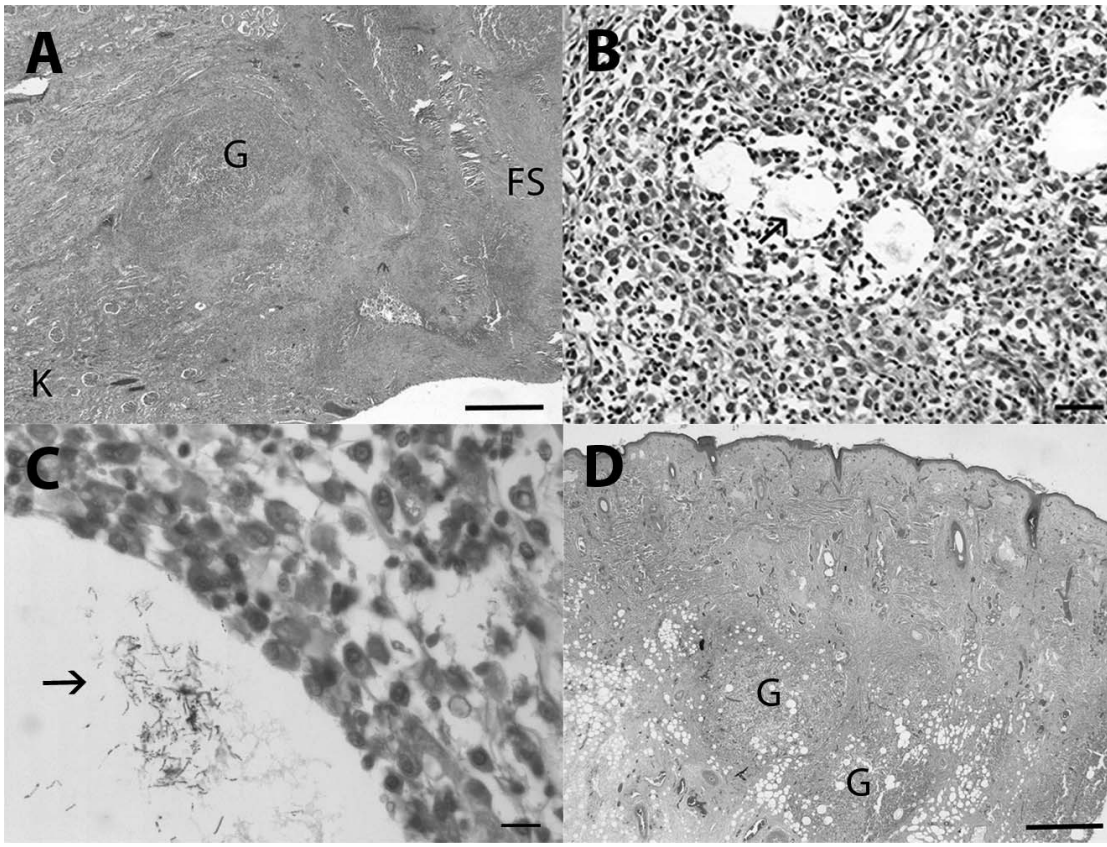

Figure 3. Histopathologic appearance of the retroperitoneal mass (**A**), left kidney (**A, B, C**), and perineal skin (**D**) in a 19-yr-old castrated male *A. binturong*. **A.** A retroperitoneal mass consisting of nodular pyogranulomatous inflammation (pyogranulomas, G) and fibrous stroma (FS) invades and expands the left renal capsule and parenchyma (K). Hematoxylin and eosin stain, scale bar = 1,000 μm. **B.** Pyogranulomatous inflammation in the left renal parenchyma. Hematoxylin and eosin stain, scale bar = 40 μm. Central clear spaces contain pale-staining bacilli (arrow). **C.** Pyogranulomatous inflammation in the left renal parenchyma, acid-fast bacilli contained within central clear spaces (arrow). Fite–Faraco acid-fast stain, scale bar = 10 μm. **D.** The perineal deep dermis and subcutis are expanded by pyogranulomas (G). Hematoxylin and eosin stain, scale bar = 1,000 μm.

viously documented in binturongs, were not identified at necropsy.[3,4,8] The mycobacterial source and the potential role of immunosuppression in the development of *M. intracellulare* infection in this binturong remain unclear.

Following skin biopsy, the binturong was treated empirically with macrolide and fluoroquinolone antibiotics. These are among the first line of therapy for mycobacterial infections in veterinary species.[6] Azithromycin and clarithromycin are considered essential to the management of pulmonary *M. intracellulare* infections in people because of substantial in vitro and in vivo activity against this bacterium.[5] Pharmacokinetic and pharmacodynamic data are not available for either marbofloxacin or azithromycin in binturongs. As such, it is unclear whether treatment with empirically derived doses of these medications may have changed the progression of disease in this animal.

This report describes an unusual case of *M. intracellulare* infection characterized by cutaneous and retroperitoneal involvement in a novel species. To the authors' knowledge, this is the first report of mycobacteriosis in a binturong. Documenting this infection is important to expand knowledge of binturong disease processes, and to demonstrate the presence of this potentially zoonotic disease in binturongs. Mycobacteriosis should be considered for binturongs presenting with recurrent pyogranulomatous dermatitis, lymphedema, and mass lesions.

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