Acute lameness in a cat with disseminated mycobacteriosis

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Summary
Mycobacterium avium infection was diagnosed in an adult cat showing acute lameness of the right hind limb, enlargement of the right popliteal lymph node and two cutaneous nodular lesions of the right chest wall. Conventional radiography of the proximal tibia showed a proliferative osteolytic lesion. Cytological examination of the right popliteal lymph node and the nodular skin lesions fine needle aspiration smears, demonstrated granulomatous inflammation with many negative staining bacilli within macrophages or in smears background. The diagnosis was confirmed by Ziehl-Neelsen staining of the smears and the identification of mycobacteria was performed by microbiological and molecular methods. Histopathology performed after the necropsy revealed disseminated mycobacteriosis with granulomatous mesenteric lymphadenitis, granulomatous pneumonia, hepatitis and tibial osteomyelitis. *M. avium* is a well-known agent of gastro-enteric, respiratory or disseminated disease in immunocompromised cats but there are few cases reported in literature of bone involvement in systemic mycobacteriosis.

A seven-year-old male neutered Birman cat was referred to a private Veterinary Clinic in Northern Italy for acute lameness of the right hind limb. The cat was kept indoor and regularly vaccinated and was negative for feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV). It had been treated with cyclosporine (2.5 mg/kg orally twice daily) for two years to control a lymphoplasmacellular enteritis.

The cat was in poor body condition showing atrophy of the right thigh muscles, pain on palpation of the right tibia and an enlargement of the right popliteal lymph node. Two firm, not painful on palpation and not ulcerated cutaneous nodular lesions of the right chest wall were also detected (Figure 1). Auscultation of the lungs revealed tachypnea and increased respiratory murmur.

A complete blood count (CBC), serum chemistry profile and protein electrophoresis analysis were performed and the results revealed leucocytosis, with increased number of mature neutrophils (14.5 x 10^3/µl, reference range 2.5-13.5 x 10^3/µl), azotemia (75 mg/dl, reference range 10-60 mg/dl) and hypoalbuminemia (2.1 gr/dl, reference range 2.3-4 gr/dl) with a decreased Albumin-to-Globulin (A/G) ratio (0.29, reference range 0.5-1.7).

Thoracic radiographs revealed a diffuse, nodular lung pattern (Figure 2), while the abdominal ultrasonography showed hepatomegaly and enlargement of mesenteric lymph nodes. Right hind limb radiographs showed a proliferative osteolytic lesion with smooth periosteal reaction in the proximal tibia metaphysis and diaphysis (Figure 3). A diagnostic hypothesis of osteomyelitis or neoplasia was made. A biopsy of the tibial lesion was performed using 16 Gauge Jamshidi-type needle and fixed in 10% buffered formalin for histopathology. Cutaneous nodular lesions and right popliteal lymph node's fine needle aspirations were performed with a 21 Gauge needle

Pending the results of the histological and cytological examinations, the cat was treated with a

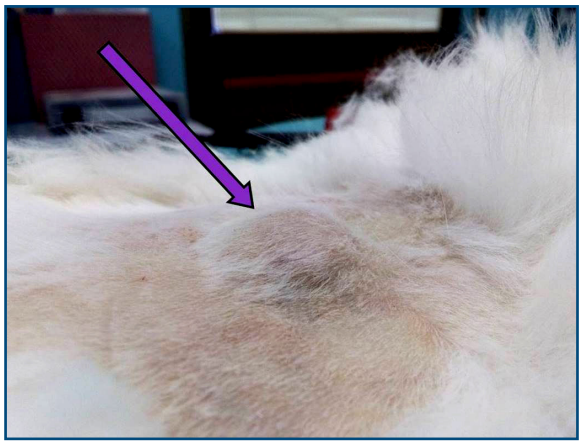

Figure 1. *Cutaneous chest wall nodular lesion indicated by the arrow.* The lesion was about 2 cm in diameter, firm, not ulcerated and not painful.

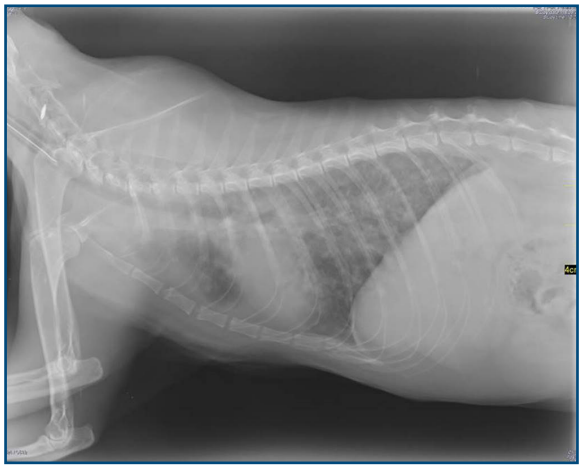

Figure 2. *Radiographic lung patterns of the seven-year-old male Birman cat.* Large pulmonary nodules and numerous smaller lung nodules, consistent with a nodular interstitial pattern scattered throughout the lungs.

nonsteroidal anti-inflammatory drug meloxicam per os at the dosage of 0.05 mg/kg /die (Metacam®).

Tibial lesion's histopathology was not conclusive due to extended cellular necrosis. Right popliteal lymph node and skin lesions' cytological examinations revealed a large number of foamy macrophages suggestive of granulomatous inflammation. The cytoplasm of the macrophages was packed with numerous small, negative-staining bacilli suggestive of mycobacteria (Figure 4). Ziehl-Neelsen stained smears demonstrated numerous small acid-fast bacilli in the cytoplasm of the macrophages confirming the diagnosis of mycobacteriosis.

Once the diagnosis of mycobacteriosis was confirmed, an additional fine-needle aspiration of the skin lesions was performed and tested using a *Mycobacterium tuberculosis* complex-specific polymerase chain reaction (IS6110 PCR) (Boniotti

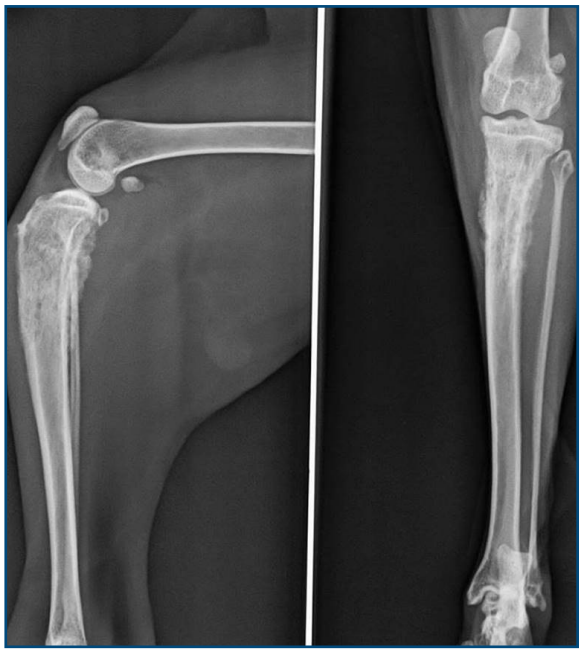

Figure 3. *Lateral and frontal radiograph of the right tibia of the seven-year-old male Birman cat.* A poorly demarcated areas of osteolysis consistent with osteolysis and smooth periosteal reaction can be observed.

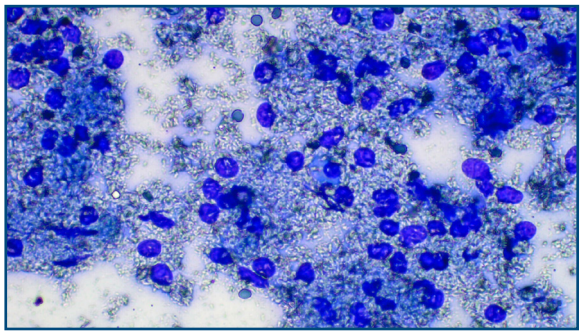

Figure 4. *Fine needle aspiration smear of skin lesion from the seven-year-old male Birman cat with* Mycobacterium avium *infection.* Large numbers of small, negative-staining bacilli may be seen within macrophages or in smear backgrounds. Diff Quick-stained slide. Magnification x 40.

et al. 2014) and a nontuberculous mycobacteria (NTM) multiplex PCR assay (Kulski *et al.* 1995). Only the NTM multiplex PCR resulted positive for the genus *Mycobacterium*. The NTM was sequenced and according to the 16S rRNA gene sequence analysis, resulted 100% identical to *Mycobacterium avium*.

M. avium was also isolated on culture from the same pathological samples and identified by PCR and sequencing as reported above.

During the diagnostic pathway which lasted about seven days, the cat's clinical conditions got worse, showing asthenia, icterus, dehydration and

hypothermia (37.1 °C). Tachypnea and discordant breathing effort were also detected.

The CBC, serum chemistry profile and protein electrophoresis analysis were repeated. The most significant results were characterized by a severe leucocytosis (35.3 x 10^3/µl, reference range 5.5-19 x 10^3/µl) with neutrophilia (34.95 x 10^3/µl, reference range 2.5-13.5 x 10^3/µl), lymphopenia (0.35 x 10^3/µl, reference range 1.5-7 x 10^3/µl) and hyperbilirubinemia (1.61 mg/dl, reference range 0.1-0.5 mg/dl).

The cat was hospitalized and oxygen was administrated with a nasal catheter in an oxygen cage. Treatment included fluid therapy (Ringer's lactate solution), marbofloxacin (Marbocyl®) 2 mg/kg daily intravenously, clarithromycin (Klacid®) 10 mg/kg twice a day orally and ceftriaxone (Rocefin®) 10 mg/kg twice a day intravenously. After few days of therapy the cat died.

At the necropsy, several greyish caseous nodules,

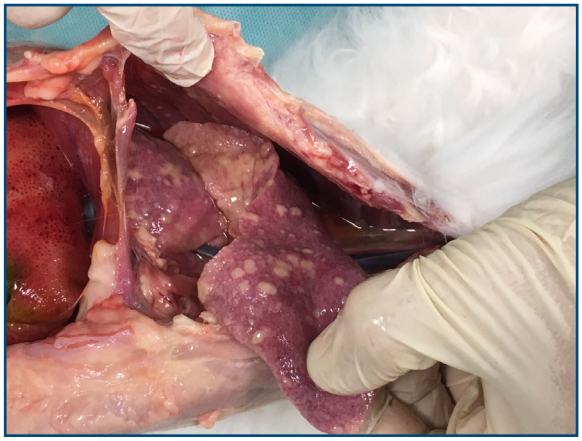

Figure 5. *Right lung (apical lobe) of the seven-year-old male Birman cat.* Greyish nodules, of about 0.3-0.5 cm of diameter are disseminated throughout the lung parenchyma.

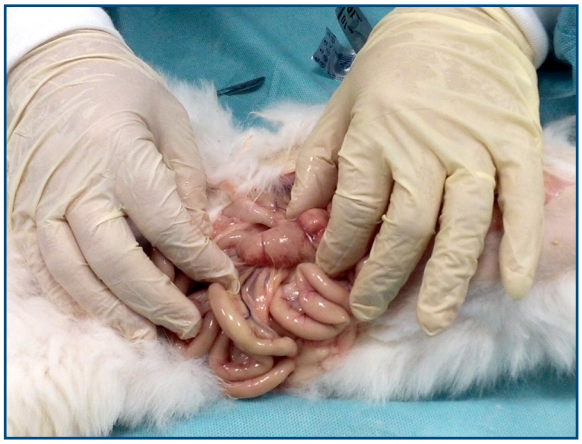

Figure 6. *Mesenteric lymph nodes of the seven-year-old male Birman cat.* Severe enlargement of mesenteric lymph nodes.

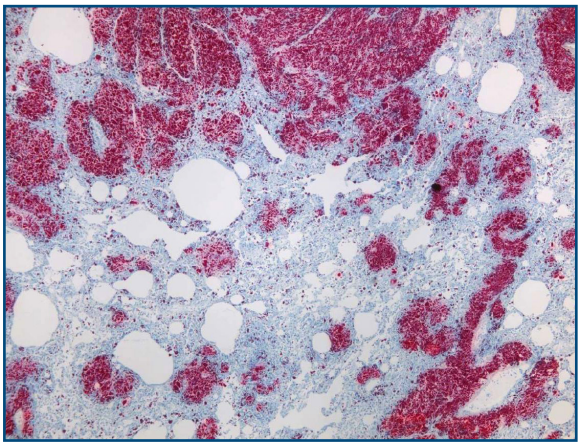

Figure 7. *Photomicrograph of the lung of the seven-year-old male Birman cat.* Acid-fast staining revealed numerous positive bacilli inside macrophage cytoplasm. Ziehl-Neelsen stain. Magnification x 4.

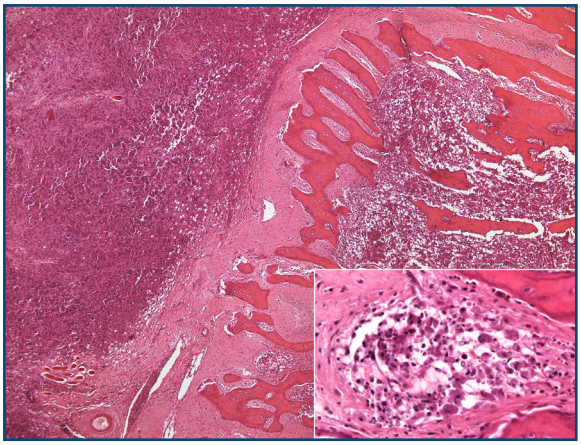

Figure 8. *Photomicrograph of the Birman cat's tibial bone.* Histopathology revealed granulomatous reaction with areas of necrosis, presence of epithelioid cells and fibroblasts surrounding bone trabeculae. In the inset a higher magnification of a granulomatous lesion is depicted. Haematoxylin-Eosin. Magnification x 4; inset x 20.

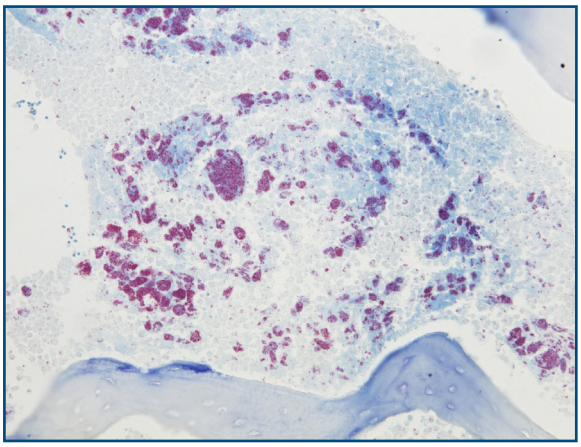

Figure 9. *Photomicrograph of the Birman cat's tibial bone.* Numerous acid-fast bacilli in the cytoplasm of macrophages can be observed. Ziehl-Neelsen stain. Magnification x 20.

of about 0.3-0.5 cm of diameter were disseminated throughout the lung parenchyma (Figure 5). Lymphadenomegaly of the mesenteric lymph nodes and hepatomegaly were also observed (Figure 6). Histopathology revealed granulomatous pneumonia, hepatitis, lymphadenitis, nephritis and tibial granulomatous osteomyelitis and Ziehl-Neelsen staining confirmed the mycobacterial infection in all these tissues (Figures 7, 8, and 9).

Clinical presentation of mycobacterial infection in cats is variable and depends on the species of mycobacteria involved and on the route of infection (Gunn-Moore 2010).

Mycobacterial infection in domestic cats can cause different clinical syndromes: tuberculosis (TB), feline leprosy syndrome (FLS) and non-tuberculous mycobacteriosis (NTM).

Feline TB is caused by *M. bovis* or *M. microti* (O'Halloran *et al.* 2019, Major *et al.* 2016), while infection with *M. tuberculosis* in cats is very rare, probably because they are naturally resistant to the infection (Smith 1965). In most cases of feline TB cutaneous lesions are observed, but some affected cats can develop systemic signs (Gunn-Moore 2014). Feline leprosy syndrome is caused by *M. lepraemurium* and other similar bacteria as *M. visibile* and *Mycobacterium* sp. Tarwin (O'Brien *et al.* 2017, Torii *et al.* 2016). Feline leprosy syndrome is primarily a cutaneous disease, nevertheless the systemic infection can be observed (Gunn-Moore 2014). Non-tuberculous mycobacteriosis is caused by facultative pathogenic opportunistic saprophytes mycobacteria from soil, water and vegetation such as *M. fortuitum* and *M. avium*-intracellulare complex (Kanegi *et al.* 2019, Knippel *et al.* 2004, Krajewska-Wędzina *et al.* 2019). *M. avium* belongs to non-tuberculous mycobacteria and typically causes disease in birds, but it also can infect other animal species (Thorel *et al.* 2001).

Skin lesions represent the most common clinical presentation in cats, typically affecting the 'fight and bite sites' such as head, extremity of the limbs, tail base or perineum. In particular, *M. avium* infection risk in cats is mainly related to wound contamination by mycobacteria present in the environment, while entry through the gastrointestinal or respiratory tracts is considered more rare (Gunn-Moore 2014). *M. avium* is less frequently reported in mycobacteriosis in cats than other mycobacteria and is usually an agent of opportunistic infections (Gunn-Moore *et al.* 2011). Peripheral lymphadenopathy, digestive disorders and respiratory signs are usually observed in *M. avium* infection in cats (Baral *et al.* 2006, de Groot *et al.* 2010) while bone's involvement is rare (Bennett *et al.* 2011, Lo *et al.* 2012, Lalor *et al.* 2017). Disseminated mycobacteriosis in cats is usually associated to immunodeficiency caused by concomitant infections (e.g. FIV and FeLV) or immunosuppressive treatments. Nevertheless four cases of mycobacteriosis in immune competent cats are described in literature (Lalor *et al.* 2017).

In our clinical case, at the onset of the clinical symptoms, the cat was under treatment with cyclosporine (Neoral®) 2.5 mg/kg orally twice daily. The therapy started two years before to control a lymphoplasmacellular enteritis. Cell-mediated immune response (CD4+ T lymphocytes and CD8+ T lymphocytes) plays a key role against mycobacterial infections. Cyclosporine induce a decrease in function of T lymphocytes through calcineurin inhibition, thus in this clinical case it can be supposed that the decrease in function of T lymphocytes may have caused the spread of *M. avium*, leading to systemic infection. A case of granulomatous osteomyelitis of the coxofemoral joint in a cat treated for a long time with cyclosporine for kidney transplant is described (Lo *et al.* 2012).

Lameness in mycobacteriosis is an unusual clinical presentation. The most common radiological features in mycobacterial bone infection in cats are osteolytic lesions, most typically permeative osteolytic lesion with soft tissue oedema and lymph node hyperplasia (Bennett *et al.* 2011).

Major and colleagues (Major *et al.* 2016) reported computed tomographic (CT) findings of skeletal lesions in 7 cats with confirmed mycobacterial disease and Lalor and colleagues (Lalor *et al.* 2017) described 4 clinical cases of cats with joint-associated tuberculosis, probably due to an infected rodent bite close to or into the affected joint. Considering these data, mycobacterial bone involvement in cats should always be considered in the differential diagnosis when clinical, radiological and echotomographic signs are compatible with osteomyelitis.

In the present case report the source of infection was unknown. Even if the cat was kept indoors most of the time, contamination from the environment appears to be most likely. The infection may have been acquired by inhalation, considering the presence of severe lung lesions, or by ingestion, considering the involvement of the mesenteric lymph nodes. *Mycobacteria* may have also spread from the cutaneous lesions haematogenously to the other organs, including the tibia (Baral *et al.* 2006, Barry *et al.* 2002, de Groot *et al.* 2010, Jordan *et al.* 1994, Rivière *et al.* 2011).

The prognosis of mycobacteriosis can be variable, depending on the type of mycobacteria involved, and on the infection's extent and severity. Disseminated *M. avium* complex infections usually respond poorly to treatment and are associated with a poor prognosis. Recommended first-line therapy is clarithromycin with clofazimine (4-8 mg/kg daily) or rifampin as reported in few cases with good

outcomes (Lloret *et al.* 2013). There are rare reports of cats with *M. avium* infection being successfully treated using combination antibiotic therapy including rifampin, enrofloxacin, and clarithromycin (Greene and Gunn-Moore 1990, Lemarie 1999). In the present case marbofloxacin, claritromicin and ceftriaxone were used to limit the potential hepatotoxicity of rifampicin. Therapeutic failure was probably due to the cat's immunosuppression, the multiple organ infection, the late diagnosis and the use of an incorrect association of antibiotics.

Mycobacterial infections are recognised as a global health concern in humans, domestic and wild animals. Wild and free-ranging domestic ungulates are the main reservoirs in nature of *M. bovis* and carnivore wild populations are generally considered spill-over hosts that become incidentally infected. *M. avium* infections have also been reported in wild animals, but clinical disease induced by this agent is considered more rare (Biet *et al.* 2005).

Mycobacterial infections have been reported both in free-living felids and in those kept in captivity.

Free-living carnivores generally acquire the infection through predation (Biet *et al.* 2005). The administration of contaminated food has also been described as a possible source of infection in wild felids in captivity, especially if they are confined and have reduced immune function due to the stress associated with their captive condition (Lantos *et al.* 2003).

Mycobacterial infections in wild carnivores reported in literature mainly concern *M. bovis* infections in free-living African felids (Viljoen *et al.* 2015). Few cases of mycobacteriosis have been reported in wild felids kept in captivity. Mycobacterial infection has been documented in four Asiatic lions (*Panthera leo persica*) (No authors listed, 2018), in a clouded leopard (*Neofelis nebulosa*) (Cerveny *et al.* 2013), in a Bengal tiger (*Panthera tigris*) (Cho *et al.* 2006), in a Siberian tiger (*Panthera tigris altaica*) (Lantos *et al.* 2003) and in two snow leopards (*Panthera uncia*) (Helman *et al.* 1998). Few cases of mycobacteriosis in wild native felids in Europe have also been documented (Aranaz *et al.* 2004). Briones and colleagues (Briones *et al.* 2000) reported a case of mycobacteriosis due to *M. bovis* in a free-living Iberian lynx (*Lynx pardina*) in Spain with the right elbow joint involvement. Another case was reported in two European lynxes (*Lynx lynx*) in a wild animal park in Germany (Schmidbauer *et al.* 2007).

The risk of transmission from domestic cats to humans is considered low and mainly concerns immunocompromised people (Rivière *et al.* 2011), however its zoonotic potential should not be underestimated. Typing mycobacteria is of fundamental importance because infections caused by NTM are indistinguishable from tuberculosis-associated infections, so identification and correct handling of potential cases is of the upmost importance. Pet owners must be made aware of the potential zoonotic risks, as well as the costs and possible complications of treatment. Particular caution should be taken if the household contains any potentially immunocompromised members, or if the animal has generalized disease, respiratory tract involvement, or extensive draining cutaneous lesions. In these cases, euthanasia should be seriously considered.

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