Primary Pulmonary Mycobacteriosis in a Cat: Immunohistochemical and Histopathological Evaluation

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Abstract: In this case report, primary pulmonary mycobacteriosis in a domestic cat was assessed using histopathological and immunohistochemical methods. The cat was admitted to a private veterinary clinic and presented with hyporexia, lethargy, and dyspnea with an abdominal component. For diagnostic purposes, thorax radiography was performed. Radiographic lung imaging showed the presence of opaque foci that were widely distributed throughout the lung. The cat's condition gradually deteriorated, and the cat died 2 hours after being brought to the clinic. Following owner consent, gross post-mortem examination was performed immediately after death at the same clinic. White foci measuring 2–10 mm in diameter covered the surface of the lungs and were present throughout the parenchyma, mostly multifocally and occasionally coalescing. No other macroscopical pathological findings were observed in other organs or on the skin. Some of the lung tissue with macroscopic lesions underwent further histopathological and immunohistochemical examination. Multifocal to coalescing areas of caseous necrosis (without a fibrous capsule) with karyorrhectic nuclei, and numerous macrophages were observed. Ziehl-Neelsen staining revealed many acid-fast bacteria. Immunohistochemical staining revealed positive immunostaining for Mycobacterium spp. This case study highlights the importance of considering zoonotic risks in cats diagnosed with primary pulmonary mycobacteriosis.

Introduction

Mycobacteria are nonsporulating, acid-fast, weakly Gram-positive, and straight to slightly curved bacilli that measure 0.3–0.6 µm in width and 1–4 µm in length (1-4). They are associated with local and disseminated infections in a range of host species (1-4) and include the Mycobacterium avium and Mycobacterium tuberculosis complexes as well as many other nontuberculous species. The M. tuberculosis complex includes M. tuberculosis, M. bovis, and M. microti (1, 4, 5).

Mycobacteria are phagocytosed by macrophages after entering the body through the alimentary tract, lungs, and skin. In the event of an insufficient cell-mediated immune response, they can persist and replicate within phagocytes by inhibiting phagosome-lysosome fusion, among other defence mechanisms. Mycobacteria cause some of the most difficult and significant infections in the world due to these defence mechanisms (6, 7).

Granulomatous inflammation occurs not only in cases of tuberculosis but also in other mycobacterial infections (8). Mycobacteriosis is usually characterized by a proliferative lesion type, in contrast to the rarely observed exudative lesion type (9). Exudative lesions, in which neutrophils are the major cell type, are both triggered by and induce high local bacillary load and tend to enlarge and progress toward liquefaction and cavitation. By contrast, proliferative lesions are triggered by low bacillary loads, mainly comprise epithelioid cells and fibroblasts, and tend to fibrose, encapsulate, and calcify (10).
Infected cats typically present with localised cutaneous disease with possible local lymph node involvement, which may become systemic, but can also present with primary gastrointestinal or respiratory disease as well as other primary disorders (11-16). Pulmonary involvement most likely arises via haematogenous spread from distant sites to the lungs, and pulmonary lesions are typically interstitial and extensive, causing dyspnoea and rarely progressing to coughing (4, 17).

Mycobacterial infections are recognised as a global health concern, both for humans and animals (18-20). One species known to be infected by a range of different mycobacteria is the domestic cat (12). Mycobacterial disease in domestic cats can cause many different syndromes, including tuberculosis, feline leprosy, and nontuberculous mycobacteriosis caused by nontuberculous mycobacteria (4, 21-24). A study in Great Britain included 339 cases of mycobacterial diseases in cats, from which the following bacteria were cultured: *M. microti* (19%), *M. bovis* (15%), *M. avium* (7%), and non- *M. avium* nontuberculous mycobacteria (6%); no bacterial growth in culture occurred in 53% of the cases (12). Different species cause feline mycobacterial infections, including *M. avium*, *M. tuberculosi*, *M. bovis*, and *M. microti*, of which the latter two are the most important (25, 26).

This report describes a case of primary pulmonary feline mycobacteriosis using histopathological and immunohistochemical techniques. Furthermore, this report aims to attract the attention of veterinarians and cat owners regarding this disease with zoonotic potential in terms of public health.

Case presentation

A 2-year-old, neutered, female, domestic shorthair Mackerel tabby cat was presented to a private veterinary clinic with reported hyporexia, lethargy, and dyspnea with an abdominal component. According to the anamnesis, the cat had no previous chronic disease or outdoor access, and there was no other cat in the house. For diagnostic purposes, thoracic radiography was performed. Multifocal ill-defined and sometimes confluent lesions with soft tissue opacity were widely distributed throughout the lung parenchyma (Figure 1A). While the cat was being treated for respiratory problems, its condition gradually deteriorated, and it died 2 hours after being brought to the clinic. Following owner consent, gross post-mortem examination was performed immediately after death at the same private veterinary clinic. Macroscopic examination revealed firm white foci measuring 2–10 mm in diameter on the surface of the lung and throughout the parenchyma, which occasionally coalesced over larger extensive areas (Figure 1B). The trachea lumen was filled with a foamy serous liquid. No gross pathological findings were observed in other organs or skin. After identifying macroscopic lesions in the lung, a sample of the fresh lung tissue was submitted to the Department of Pathology, Faculty of Veterinary Medicine, Selçuk University, for further histopathological examination.

Five samples of the lesioned lung tissue were fixed in 10% neutral buffered formaldehyde solution. After routine histopathological tissue processing, tissues were embedded in paraffin and cut into 5-µm-thick sections. These sections underwent haematoxylin and eosin and Ziehl-Neelsen staining (27) and immunohistochemical staining with an anti-*M. tuberculosis* antibody (1:100, Abcam, ab214721) in a staining device (Leica, Bondmax) using the BondTM polymer refine detection system (Leica DS9800) according
Figure 2: Histopathological and immunohistochemical findings of the lungs. A: Multifocal and coalescing nodular areas consisting of caseous necrosis without fibrous capsules (asterisk) and oedemas (arrows). Haematoxylin-Eosin staining; scale bar: 200 µm. B: High magnification of nodular lesion: caseous necrosis (asterisk), mineralisation (arrowheads), and lymphohistiocytic cell infiltration. Haematoxylin-Eosin staining; scale bar: 50 µm. C: Necrotic areas (asterisk) and numerous macrophages (arrows) around the vessel and necrotic vasculitis. Haematoxylin-Eosin staining; scale bar: 100 µm. D: Acid-fast bacteria in areas of caseous necrosis. Ziehl-Neelsen staining; scale bar: 20 µm. E: Positive immunostaining for Mycobacterium spp. in areas of caseous necrosis. IHC; scale bar: 100 µm. F: High magnification of Mycobacterium spp. immunostaining. IHC; scale bar: 20 µm.
to a previously reported method (28). Sections treated with phosphate buffer saline instead of primary antibodies served as negative controls. Slides were examined by light microscopy (Olympus BX51, Tokyo, Japan) and photographed (Olympus EP50, Tokyo, Japan).

Multifocal to coalescing nodular areas consisting of caseous necrosis with karyorrhectic nuclei were widely observed in the lung. Around the necrotic areas, no fibrous capsules were observed but many lymphohistiocytic cell infiltrations and alveolar oedema were seen (Figure 2A). Mineralisation was observed in the areas of caseous necrosis (Figure 2B). Around the vessel, similar necrotic areas and necrotic vasculitis were observed (Figure 2C). Local haemorrhagic areas were also identified. Although there were very few epithelioid macrophages, no giant cell types were found. In the areas of caseous necrosis and in the cytoplasm of macrophages, Ziehl-Neelsen staining revealed numerous acid-fast bacilli (Figure 2D), and anti-M. tuberculosis immunostaining was positive (Figure 2E, F).

**Discussion**

This case report presents primary pulmonary mycobacteriosis in a domestic cat diagnosed using histopathological and immunohistochemical techniques. Radiography can detect pulmonary involvement in many cases of mycobacteriosis; however, the radiographic appearance of lesions is variable and nonspecific. In feline mycobacteriosis, pulmonary involvement is commonly interstitial, but may progress to bronchial changes in chronic cases (17). In the current case, diffuse interstitial changes were observed.

Mycobacterial lesions in carnivores differ from those in other species. Typical tuberculous granulomas are not as common, and when they occur, caseous necrosis is not a prominent gross feature. More often there is nonspecific granulation tissue in which macrophages are scattered at random, and giant cells are rare or absent (29). Necrosis associated with feline mycobacteriosis is called caseous necrosis and is defined as areas where cellular and structural details are lost due to the accumulation of eosinophilic and basophilic (karyorrhectic) residues (30). In the current case, mycobacterial lesions displayed caseous necrosis, in which karyorrhectic nuclei were observed, whereas giant cells were not. Few epithelioid macrophages and no evidence of a fibrous capsule were observed, which suggests a more acute infection. Macrophagic lesions were only observed in the lungs, which indicates that the route of infection was via inhalation of infectious particles.

The current case was of a domestic cat without access to the outdoors or other animals, indicating the possibility of human-to-animal transmission. Mycobacterial diseases are considered both zoonanthroponotic and zoonotic (31). If an immunocompromised individual has an active untreated mycobacterial infection, there is a potential risk of transmission to others, including cats (32). There is a theoretical risk of transmission of infection from cats to humans via infectious aerosols. However, if the cat has untreated productive respiratory disease, the greatest risk to humans is exposure to infectious fluids (33). In the current case, the presence of respiratory tract symptoms increased the zoonotic risk. In terms of public health, cats infected with mycobacteria should be examined, and necessary measures should be taken.

**Conclusions**

This study characterized the radiographic, pathological, and immunohistochemical appearance of primary pulmonary mycobacteriosis lesions in a cat. The purpose of this case report was to draw attention to feline mycobacteriosis, which is under-reported but increasingly recognised. Suspected or infected cats with respiratory symptoms should be examined on a regular basis because they can pose a zoonotic risk to cat owners, children, and veterinarians.

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**References**

Nekatera dele pljučnega tkiva z makroskopskimi spremembami smo dodatno histopatološko in imunohistokemično fokalno in mestoma zlivajoče. V drugih organih in na koži ni bilo opaznih drugih makroskopskih patoloških sprememb.

Žarišča, premera od 2 do 10 mm, so prekrivala površino pljuč. Prisotna so bila po celotnem parenhimu večinoma multi je pokazalo pozitivno imunobarvanje za in številnimi makrofagi. Ziehl-Neelsenovo barvanje je razkrilo številne acidofilne bakterije. Imunohistokemično barvanje pregledali. Opazili smo multifokalna do zlivajoča se območja kazeozne nekroze (brez fibrozne kapsule) s karioznimi jedri.

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Ključne besede: mikobakterioza mačk; imunohistokemija; histopatologija; pluča