IDIOPATHIC STERILE PYOGRANULOMA IN THREE DOMESTIC CATS

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Abstract

Pyogranulomatous inflammation has been extensively described in cats, in particular in cases of feline infectious peritonitis (FIP) and also associated with Mycobacteria, Actinomyces, Nocardia, Rhodococcus, and fungal infections. Idiopathic sterile pyogranulomatous dermatitis has also been described. In this case series we describe the clinical presentation, histopathology, and outcome of three cases of feline idiopathic sterile steroid-responsive pyogranuloma with different presentation and different locations of the lesion, but with the common feature of having a mass with no superficial skin involvement. To the authors' knowledge the presence of sterile pyogranuloma in the absence of skin involvement has not been described previously in the cat.

Introduction

Pyogranulomatous inflammation (PI) is a chronic inflammatory lesion characterised by a predominance of macrophages and neutrophils, often in combination with plasma cells and giant cells (Raskin.,2001). Generalised PI has been extensively described in the veterinary literature, particularly in cases of feline infectious peritonitis (FIP) (Weiss et al., 1981; Kipar et al.,2005). Cutaneous, subcutaneous and more rarely systemic PI in cats is mainly associated with mycobacteria and fungal infections (Malik et al., 1992; Brömel and; Malik et al., 1994; Malik et al.,2000; Baral et al.,2005; Sykes 2005;) with sporadic implication of Actinomyces, Nocardia, Rhodococcus, Streptomyces, Francisella, Bartonella and Leishmania (Patel, 2002; Valentine et al.2004; Malik et al., 2006; Santero et al.,2008; Sharmanet al.,2009; Varanat et al.,2012;Traslavina et al.,2015;. Idiopathic sterile pyogranulomatous dermatitis has also been described in cats (Scott et al., 1990). We describe the clinical presentation and outcome of three cases of feline idiopathic sterile pyogranuloma with different presentation and locations, but with the common picture of a mass with no superficial skin involvement. To the authors' knowledge the presence of sterile

pyogranuloma with mass appearance and absence of skin involvement has not previously been described in cats.

Case 1

A 5-year old male neutered domestic short hair (DSH) cat was presented for investigation of a right sided naso-maxillary mass. Generalised gingival inflammation, more severe on the right canine tooth, was noticed at a routine medical check. A dental polish, descale and a right canine root extraction was performed by the referring veterinarian. The facial swelling appeared 2 weeks following the dental procedure and the cat was treated with 6 weeks' of amoxicillin-clavulanic acid without improvement. The owner did not report any clinical signs apart from occasional sneezing during the past few weeks. On physical examination the patient was bright and alert. There was a firm, poorly defined mass extending from the right proximal maxilla to the nasal region causing facial deformation. No ulceration or skin lesions were present, oral examination was unremarkable except for a swelling on the right side of the proximal maxilla, close to the area of previously removed canine tooth. Complete blood count (CBC) and biochemistry were unremarkable, FELV antigen and FIV antibody tests were negative. Oral radiography rule out the presence of a remaining canine tooth root. Due to the high suspicion of neoplasia a CT scan of head, neck, and thorax was performed. The CT scan confirmed the presence of a soft tissue mass on the nasal dorsum extending laterally to the missing canine tooth and causing lysis of the incisive and nasal bones. The mass extended in to the right nasal cavity with destruction of the nasal turbinates and mild deviation of the nasal septum (Fig 1). Surgical wedge biopsies were taken after incision and blunt dissection of the skin and subcutis until visualization of the mass. The biopsies were taken in two different sites within the mass to increase the chances of collecting a representative sample and were immediately submitted for histopathology. Due to the high suspicion of neoplasia, tissue samples for culture were not taken and no antibiotics or other types of treatment were

started. Microscopic examination exhibited a severe pyogranulomatous and lymphocytic cellulitis and a mild pyogranulomatous and lymphocytic myositis (Fig.2a,2b). There was no evidence of neoplasia in any of the samples examined. No infectious etiological agents could be detected following Gram, Ziehl Nielsen (ZN), and Periodic Acid Schiff (PAS) staining. Immunohistochemistry for coronavirus FIPV3-70 anti-feline coronavirus antibodies (Tammer et al., 1995) was negative. The sample was sent for pan-fungal (ITS1-ITS2 region of ribosomal DNA) as previously published (Lau et al., 2007) and mycobacteria PCR assay (mycobacterial ITS1-ITS2 regions) on the paraffin block sample as previously published, (Hughes et al., 1997; Fyfe et al., 2008) both of which were negative. Bacterial 16s RNA probe FISH analysis for eubacteria was negative (Maunder et al., 2016). On clinical examination the mass remained of similar size for the following 2 months, however the sneezing became more frequent. Prednisolone treatment was started at 1 mg / kg daily orally for and the mass reduced in size and the sneezing markedly improved, the same dose of prednisolone was continued for 12 months until last follow up. The cat was still alive at the time of writing 12 months after presentation and the facial swelling and the sneezing resolved almost completely.

Case 2

A 6-year old female neutered outdoor DSH was presented for further investigation of an abdominal mass. The referring veterinary surgeon detected the mass as an incidental finding when the cat presented for annual booster vaccination. The patient did not have any history of previous illness and the owner did not reported any current problems or clinical signs, the cat was otherwise fit and well. On abdominal palpation a non-painful, firm, partially mobile mass was present in the mid-caudal abdomen. At this stage, considering the size of the mass in the abdomen and the clinical presentation, the most likely diagnosis was neoplasia, however FIP, fungal and mycobacterial granuloma were also considered. CBC and biochemistry were

unremarkable apart from mild hyperglobulinaemia 54 g/l (ref range 24-47). FIV antibody and FELV antigen tests were negative. Abdominal ultrasound showed a heterogeneous mass with mixed echogenicity and no other abnormalities. The mass measured approximately 5x4 cm, and was thought to originate from the mesenteric lymph node. Cytology of fine needle aspirates (FNAs) of liver and spleen taken to rule out the possibility of related disease in those organs was unremarkable.. An FNA of the mass revealed pyogranulomatous inflammation with a mixed mainly neutrophils and macrophages infiltrates. An ultrasound-guided Tru-cut biopsy of the mass was obtained under general anaesthesia, which confirmed the lesion to be pyogranulomatous. In view of the continued high suspicion of neoplasia, an exploratory laparotomy was performed. The mass was confirmed to originate from the mesenteric lymph node and could not be resected without damage to the intestinal blood supply. Multiple wedge biopsies were obtained to submit for culture and histopathology. Histopathological examination showed a pyogranulomatous and necrotising lymphadenitis, with fibrosis. No etiological agents were visible on Gram, ZN, and PAS staining. Immunohistochemistry for coronavirus (FIPV3-70 anti-feline coronavirus antibodies) was also negative. The sample was sent for pan-fungal PCR (ITS1-ITS2 region of ribosomal DNA) on paraffin tissue block and mycobacteria PCR (Mycobacterial ITS region) on frozen sample that were both negative. Bacterial 16s RNA probe FISH analysis for eubacteria was negative. Due to the incidental finding and the asymptomatic nature of the mass it was decided to only monitor the mass and no treatment was given. The mass remained of similar size and no clinical signs related to the mass were ever reported. The cat was still alive and well almost 2 years after diagnosis when she died in a road traffic accident.

Case 3

A 6-year old male neutered DSH was presented for further investigation of a 5-6 cm right submandibular subcutaneous mass. The mass was first noticed by the owner 5 months

previously when it was approximately 3 cm diameter, the mass was not painful with no associated clinical signs. The patient was treated by the referring veterinarian with a week course of meloxicam and amoxicillin clavulanic acid with no improvement. On presentation the cat was still clinically well and the owner did not report any problems or clinical signs aside of the slow growing mass in the submandibular area. The mass was suspected to be neoplastic and thoracic radiography and a wedge biopsy sample were taken under general anaesthesia. Thoracic radiography showed focal mineralization of the left caudal lung lobe, considered an incidental finding but was otherwise unremarkable. The histopathological diagnosis was marked pyogranulomatous and lymphocytic cellulitis with mild myositis and no evidence of neoplasia. The mass continued to grow and, neoplasia remained high on the differential list, so the mass was surgically resected. The second histopathology report confirmed the previous diagnosis of a pyogranuloma, and no aetiological agents could be found with PAS, Gram, and ZN staining. PCR and culture for Mycobacteria were performed on frozen (stored at -80) and fresh tissue respectively and were both negative. A pan-fungal PCR (ITS1-ITS2 region of ribosomal DNA) on paraffin block tissue was also negative except for isolation of "*Candida albicans*". Although this was most likely a contaminant, the patient was prescribed a 3-month course of itraconazole. One month later new numerous nodules non-associated to the previous removed mass were found in the lymphatic chain of the neck, together with an enlarged prescapular lymph node, no regrowth of the previous removed granuloma was present. An MRI confirmed the presence of the nodules in the neck and an enlarged right prescapular lymph node; no other abnormalities were found. Radiography of the thorax and abdominal ultrasound were unremarkable. The prescapular lymph node and three nodules in the neck were removed for diagnostic purpose. Histopathology confirmed the presence of pyogranulomatous cellulitis and myositis, with mild dermal fibrosis and pyogranulomatous lymphadenitis. No aetiological agents could be found despite repeated

examination of PAS, Gram, and ZN stains. Tissue culture, panfungal and mycobacteria PCR were again negative. Immunohistochemistry for coronavirus antigen (FIPV3-70 anti-feline coronavirus antibodies) and 16s RNA probe FISH analysis for eubacteria were performed and were both negative. The lesions remained unchanged for the following 3 weeks and prednisolone was prescribed at 1 mg /kg daily for 2 weeks but with no improvement. The dose was increased to 3 mg/kg for the following 2 weeks, reducing to 2 mg/kg for the following month. Despite the initial lack of response, the lesions markedly improved and completely disappeared over the following two months. The prednisolone was gradually reduced and stopped. At the time of writing the cat was still asymptomatic and no recurrence of the lesions has been documented, more than one year after initial presentation.

Discussion

To the authors' knowledge this is the first report of a feline idiopathic sterile pyogranuloma without skin involvement. The pyogranulomas here described affected young to middle aged DSH cats, they had different locations, but similar histopathological features with no etiological agents found, despite extensive investigations. The 16s rRNA PCR for Mycobacteria Spp. in fresh, frozen or even in paraffin embedded tissue sample is considered a very sensitive and specific test for diagnosis of lepromatosis/mycobacteriosis in cats compared to histopathology and ZN staining, so the possibility of a false negative is unlikely (Hughes et al., 2004). The panfungal PCR amplification of the ITS1-5.8s-ITS2 region of ribosomal DNA is considered a sensitive test for detection of fungi and in combination with conventional laboratory tests, it improves the accuracy of fungal detection in tissue specimen (Lau et al., 2007), so a fungal infection was considered extremely unlikely. All the formalin

fixed and paraffin embedded tissue samples for PCR were fixed for a maximum of 24 hours and analysed within 7 days from sample collection, limiting false negative results as previously published (Hughes et al.,2004; Reppas et al.,2013). On the 16s RNA probe FISH analysis no bacteria could be found. This test is considered more sensitive than culture in the detection of bacteria, ruling out the possibility of a bacterial involvement. Bartonella is often asymptomatic in cats, however recently Bartonella has been isolated in pyogranulomatous myocarditis in 2 cats (Varanat et al.,2012). Prevalence of Bartonella in cats can be quite high and the association of Bartonella with the manifestation of any concurrent disease can be difficult to prove (Barnes et al.,2000). In our case we did not test for Bartonella, so despite unlikely we could not rule out this possibility completely. Furthermore the lack of significant progression or development of systemic lesions in particular after the treatment with prednisolone in cases one and three were more consistent with a non-infectious process. The clinical response to prednisolone in cases one and three, may in fact indicate a possible immune-mediated/inflammatory aetiology in these cases, similar to that reported in dogs (Fraga-Manteiga et al., 2016) and currently still under investigation (Bexfield et al.,2015).

In conclusion we have reported three cases of idiopathic sterile granuloma in cats with a mass appearance. The granulomas had overall a slow growth and a benign progression, despite the aggressive clinical appearance and the remarkable size of the masses in all cases. In case 3, surgical resection originally controlled the disease, however due to the development of other lesions one month later, the benefit of surgery, except for diagnostic purposes, remains unknown. Immunosuppressive treatment with prednisolone could be beneficial, however more studies need to be done to confirm this hypothesis.

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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176 **References**

177 Baral, R. M., Metcalfe, S. S., Krockenberger, M. B., Catt, M. J., Barrs, V. R., McWhirter, C., & Mitchell,
178 D. H. (2006). Disseminated Mycobacterium avium infection in young cats: overrepresentation of
179 Abyssinian cats. *Journal of Feline Medicine & Surgery*, *8*(1), 23-44.

180 Barnes A, Bell SC, Isherwood DR, *et al*. Evidence of *Bartonella henselae* infection in cats and dogs in
181 the United Kingdom. *Vet Rec* 2000; 147 (24), 673–677.

182 Bexfield, N., Myatt, P. and Gajanayake, I., (2015). Study into sterile neutrophilic-macrophagic
183 lymphadenitis in the English springer spaniel. *Veterinary Record*, *177*(4), 104-104.

184 Brömel, C., & Sykes, J. E. (2005). Epidemiology, diagnosis, and treatment of blastomycosis in dogs
185 and cats. *Clinical Techniques in Small Animal Practice*,20 (4), 233-239.

186 Farias, M. R., Takai, S., Ribeiro, M. G., Fabris, V. E., & Franco, S. R. V. S. (2007). Cutaneous
187 pyogranuloma in a cat caused by virulent Rhodococcus equi containing an 87 kb type I
188 plasmid. *Australian Veterinary Journal*, 85(1-2), 29-31.

189 Fyfe, J. A., McCowan, C., O'Brien, C. R., *et al*. (2008) Molecular characterization of a novel fastidious
190 mycobacterium causing lepromatous lesions of the skin, subcutis, cornea, and conjunctiva of cats
191 living in Victoria, Australia. *Journal of Clinical Microbiology* 46, 618-626

192 Fraga-Manteiga, E., Veloso, G.F. and Schwarz, T., (2016). Idiopathic sterile pyogranulomatous
193 lymphadenitis in a nine-month-old springer spaniel. *Veterinary Record Case Reports*, *4*(2),
194 p.e000347.

195 Hughes MS, Ball NW, Beck L-A *et al.* (1997) Determination of the aetiology of presumptive feline
196 leprosy by 16S rRNA gene analysis. *Journal of Clinical Microbiology* 35, 2464–71.

197 Hughes, M.S., James, G., Taylor, M.J., McCarroll, J., Neill, S.D., Chen, S.C.A., Mitchell, D.H., Love, D.N.
198 and Malik, R., (2004). PCR studies of feline leprosy cases. *Journal of feline medicine and surgery*, *6*(4),
199 235-243.

200 Kipar, A., May, H., Menger, S., Weber, M., Leukert, W., & Reinacher, M. (2005). Morphologic
201 features and development of granulomatous vasculitis in feline infectious peritonitis. *Veterinary*
202 *Pathology Online*, 42(3), 321-330.

203 Lau, A., Chen, S., Sorrell, T., Carter, D., Malik, R., Martin, P. and Halliday, C., (2007). Development and
204 clinical application of a panfungal PCR assay to detect and identify fungal DNA in tissue specimens.
205 *Journal of clinical microbiology*, *45*(2),380-385.

206 Malik, R., Wigney, D. I., Dawson, D., Martin, P., Hunt, G. B., & Love, D. N. (2000). Infection of the
207 subcutis and skin of cats with rapidly growing mycobacteria: a review of microbiological and clinical
208 findings. *Journal of Feline Medicine and Surgery*, *2*(1), 35-48.

Malik, R., Hunt, G. B., Goldsmid, S. E., Martin, P., Wigney, D. I., & Love, D. N. (1994). Diagnosis and treatment of pyogranulomatous panniculitis due to Mycobacterium smegmatis in cats. *Journal of Small Animal Practice*, *35*(10), 524-530.

Malik, R., Wigney, D. I., Muir, D. B., Gregory, D. J., & Love, D. N. (1992). Cryptococcosis in cats: clinical and mycological assessment of 29 cases and evaluation of treatment using orally administered fluconazole. *Journal of Medical and Veterinary Mycology*, 30(2), 133-144.

Malik, R., Krockenberger, M. B., O'BRIEN, C. R., White, J. D., Foster, D., Tisdall, P. L. C., ... & Howe, J. (2006). Nocardia infections in cats: a retrospective multi-institutional study of 17 cases. *Australian veterinary journal*, *84*(7), 235-245.

Maunder, C.L., Reynolds, Z.F., Peacock, L., Hall, E.J., Day, M.J. and Cogan, T.A. (2016), *Campylobacter* Species and Neutrophilic Inflammatory Bowel Disease in Cats. J Vet Intern Med, 30, 996–1001

Oort, J. and Scheper, R.J., (1976). Histopathology of acute and chronic inflammation. *Agents and actions. Supplements*, (3), 25-30.

Patel, A. (2002). Pyogranulomatous skin disease and cellulitis in a cat caused by Rhodococcus equi. *Journal of Small Animal Practice*, *43*(3), 129-132.

Raskin RE (2001) General Categories of Cytologic Interpretation. In: Raskin RE, Meyer DJ. eds, *Atlas of Canine and Feline Cytology*. Saunders, Philadelphia, PA: 19–33

Reppas G , Fyfe J , Foster S et al. (2013) Detection and identification of mycobacteria in fixed stained smears and formalin-fixed paraffin-embedded tissues using PCR. *J Small Anim Pract* 54, 638–646.

Santero D, Prisco M, Ciaramella P. (2008) Cutaneous Sterile granulomas/pyogranulomas, leishmaniasis and mycobacterial infections. *J Small Anim Pract*. 49(11), 552-61.

Scott, D. W., Buerger, R. G., & Miller, W. H. (1990). Idiopathic sterile granulomatous and pyogranulomatous dermatitis in cats. *Veterinary Dermatology*, 1(3), 129-137.

Sharman, M. J., Goh, C. S., von Lande, R. G. K., & Hodgson, J. L. (2009). Intra-abdominal actinomycetoma in a cat. *Journal of Feline Medicine and Surgery*, 11(8), 701-705.

Tammer R., Evensen O., Luta H., and Reinacher M. (1995). Immunohistochemical demonstration of feline infectious peritonitis virus antigen in paraffin-embedded tissues using feline ascites or murine monoclonal antibodies. *Veterinary Immunology and Immunopathology*, 45, 177-182.

Traslavina, R. P., Reilly, C. M., Vasireddy, R., Samitz, E. M., Stepnik, C. T., Outerbridge, C., & Murphy, B. (2015). Laser Capture Microdissection of Feline Streptomyces spp Pyogranulomatous Dermatitis and Cellulitis. *Veterinary Pathology*, 52(6), 1172-1175.

Valentine, B. A., DeBey, B. M., Sonn, R. J., Stauffer, L. R., & Pielstick, L. G. (2004). Localized cutaneous infection with Francisella tularensis resembling ulceroglandular tularemia in a cat. *Journal of Veterinary Diagnostic Investigation*, *16*(1), 83-85.

Varanat M, Broadhurst J, Linder KE.,(2012) Identification of *Bartonella henselae* in 2 cats with pyogranulomatous myocarditis and diaphragmatic myositis. *Vet Pathol* 49, 608–611.

Weiss, R. C., & Scott, F. W. (1981). Pathogenesis of feline infetious peritonitis: pathologic changes and immunofluorescence. *American Journal of Veterinary Research*, 42(12), 2036-2048.

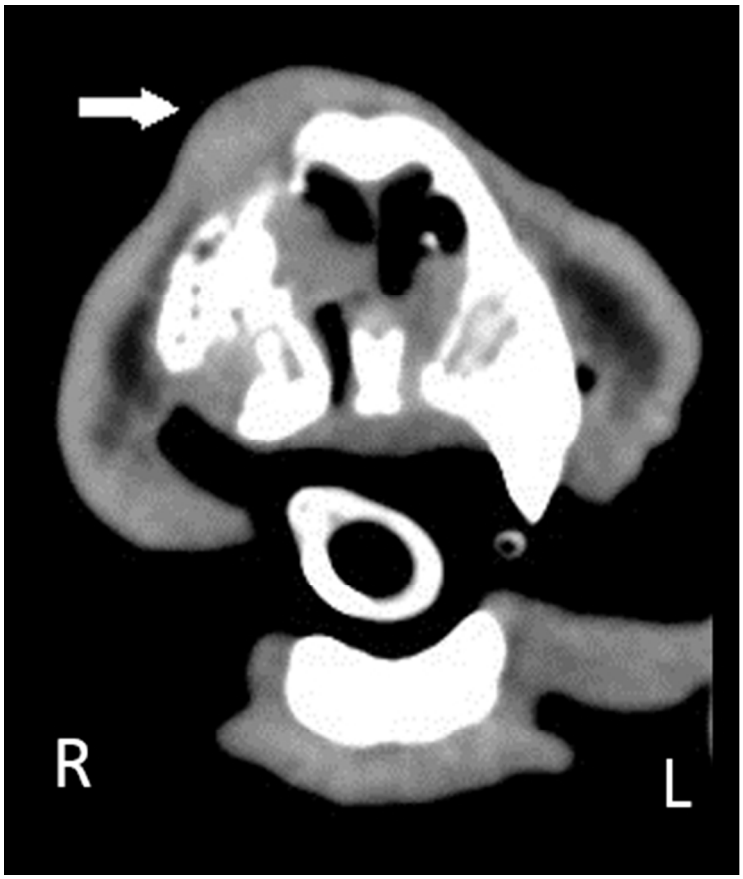

Fig.1. Case 1, 5-year old male neutered DSH cat. Transverse CT image in bone window of the nasal cavity at the level of the palatine fissures. Dorsal soft tissue density mass on the right side, causing lysis of the incisive and nasal bones and extending into the right nasal cavity without crossing midline

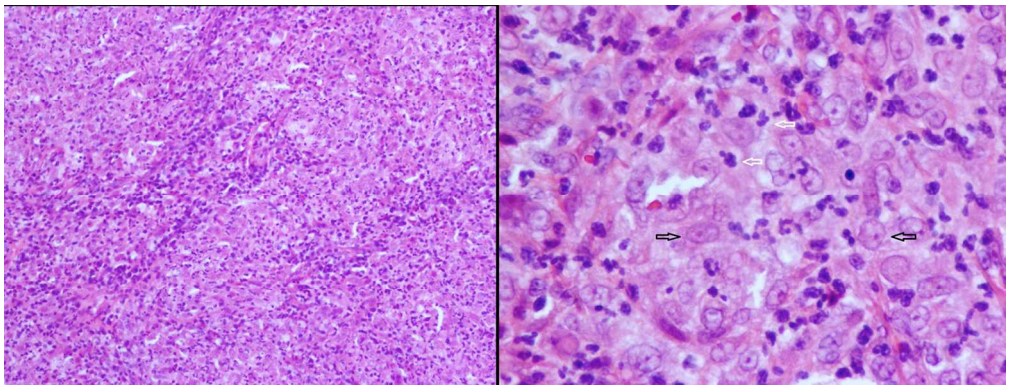

Fig 2a-b. Case 1, 5-year old male neutered DSH cat. Biopsy of subcutaneous tissue containing large numbers of macrophages, neutrophils, moderate numbers of lymphocytes and lesser numbers of plasma cells. Haematoxylin &Eosin stain x100 on the left and x400 on the right or Bar = 100 µm. White arrow neutrophils, black arrow macrophagess of plasma cells.