INFECTIOUS DISEASE

Short Title: Phycomycetes in Feline Gastrointestinal Fibroplasia

A Case of Feline Gastrointestinal Eosinophilic Sclerosing Fibroplasia associated with Phycomycetes

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Summary

Feline gastrointestinal eosinophilic sclerosing fibroplasia (FGESF) is a recently described inflammatory condition of domestic cats with unknown aetiology. A proportion of cases of FGESF are associated with bacteria, but antibiotic treatment is ineffective. It has been hypothesized that genetically predisposed cats may develop FGESF in response to the introduction of bacteria or other antigens into the intestinal wall. A 9-month-old male Persian cat presented with a history of marked acute haematemesis. A mass (10 cm diameter) was detected within the pylorus and proximal duodenum and this was not surgically accessible. On necropsy examination the duodenal wall was seen to be markedly thickened with extensive mucosal ulceration. Microscopically, there were haphazardly oriented trabecular bands of dense eosinophilic collagen, separated by wide, clear areas containing variable numbers of fibroblasts, eosinophils, mast cells, neutrophils, macrophages, lymphocytes and plasma cells. Numerous pleomorphic, non-parallel walled, sparsely septate hyphae, characteristic of phycomycetes, were present within the collagen matrix. Colonies of gram-positive and gram-negative rods were also present within the lesion. This is the first description of FGESF with intralesional fungi.

Keywords: feline gastrointestinal eosinophilic sclerosing fibroplasia; phycomycetes; cat

Feline gastrointestinal eosinophilic sclerosing fibroplasia (FGESF) is a recently described inflammatory condition of the gastrointestinal tract of domestic cats (Craig *et al.*, 2009). FGESF presents typically as an ulcerated intramural mass at the pyloric sphincter or ileocaecocolic junction. Microscopically, it is characterized by eosinophilic inflammation, large, reactive fibroblasts and trabeculae of dense collagen (Craig *et al.*,

2009). The aetiology of this condition is unknown. Craig *et al.* (2009) have suggested that it is caused by bacterial agents; however, no evidence of bacterial infection was found in almost 50% of cases reported in that study (Craig *et al.*, 2009) or in one of the few cases described later (Suzuki *et al.*, 2013). Recently, lesions resembling FGESF have been described in pumas (*Puma conclor*), where the lesions were associated with the presence of intestinal nematodes (*Cyclospirura* sp.; Eckstrand *et al.*, 2013).

A 9-month-old male Persian cat was presented for clinical evaluation with a history of acute marked haematemesis. On physical examination, the animal had pale mucous membranes and a palpable mass in the cranial abdomen. Haematological examination revealed a low packed cell volume [16.8% (reference interval: 30-45%)], neutrophilia [13.3 x 10⁹ cells/L (reference interval: 2.5-12.5 x 10⁹ cells/L)] and eosinophilia [2.7×10^9 cells/L (reference interval: 0-0.8 x 10⁹ cells/L)]. A serum biochemical profile revealed hyperglycaemia [17.0 mmol/L(reference interval: 3.3-6.7 mmol/L)] and hypoproteinaemia [total protein: 40 g/L (reference interval: 57-89 g/L)]. Radiography and ultrasonography indicated gastric distention, a thickened duodenal wall and enlarged mesenteric lymph nodes. On exploratory laparotomy a mass (approximately 10 cm in diameter) was observed within the caudal pyloric region of the stomach and proximal duodenum. The mass was considered to be inoperable and the animal was humanely destroyed.

On necropsy examination, the animal was in poor bodily condition and there was abundant perineal faecal staining. The pyloric antrum and proximal duodenum contained a mass ($8 \times 10 \times 10$ cm). In this area, the intestinal wall was markedly thickened and there were multiple broad-based, ulcerated nodules (3–4 cm diameter), protruding into the lumen (Fig. 1). Abundant fresh blood was present within the stomach and duodenal lumen. The gastrohepatic lymph nodes were markedly enlarged.

Additionally, the liver showed a moderate lobular pattern, kidneys contained multiple small cysts (up to 1 mm) and a single subdural cyst (0.5 cm diameter) was located dorsally near the junction of the cerebellum with the cerebrum. Samples of the stomach (pylorus), duodenum, kidney, spleen, liver, lung, heart and brain were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections (5 µm) were stained with haematoxylin and eosin (HE), periodic acid–Schiff (PAS), Grocott's methenamine silver (GMS) and Gram stain.

Microscopically, the intestinal mass consisted of a transmural chronic inflammatory reaction that replaced the mucosa, submucosa and partially infiltrated the muscular and serosal layers. The mass was composed of abundant, haphazardly oriented trabecular bands of dense eosinophilic and homogenous collagen, often resembling osteoid, and containing sparse numbers of large spindle-shaped cells. Between the trabeculae, wide clear areas were filled with abundant fibroblasts, eosinophils and mast cells, admixed with fewer neutrophils, macrophages, lymphocytes and plasma cells (Figs. 2 and 3). Areas of abundant granulation tissue were also present. Focally, the lesion extended to the adjacent pancreatic parenchyma. Within the trabeculae, numerous pleomorphic, sparsely septate hyphae (5–15 µm diameter) with thin non-parallel walls were observed. The hyphae branched at right angles. These hyphae stained strongly by PAS (Fig. 4) and GMS. Additionally, colonies of grampositive and gram-negative rod-shaped bacteria were also present, mainly within the area of the ulcerated mucosa.

In the adjacent gastric mucosa there was diffuse hyperplasia of the glandular tissue associated with moderate fibrosis within the lamina propria. The gastrohepatic lymph nodes had increased numbers of macrophages and contained some small granulomas with occasional multinucleated giant cells, some containing fungal hyphae.

The spleen showed abundant megakaryocytes and moderate white pulp hyperplasia. Kidneys displayed moderate interstitial nephritis and cystic dilation of tubules.

The clinical and pathological features in the present case are consistent with those described in FGESF (Craig *et al.*, 2009). Eosinophilia and involvement of regional lymph nodes are reported to occur frequently in FGESF, while involvement of the pancreas has been described at least in one case (Craig *et al.*, 2009). In the present case intralesional fungal organisms were identified.

Based on the morphology of these fungi, the differential diagnosis included zygomycosis, pythiosis and lagenidiosis (Grooters, 2003; Rakich *et al.*, 2005). These diseases are caused by phylogenetically diverse groups of pathogens, which share similar clinical and microscopical characteristics, making them difficult to distinguish (Grooters, 2003). Because of their similarity, the term 'phycomycosis' is used when the diagnosis is not confirmed by culture or molecular techniques (Grooters, 2003). While zygomycetes are considered true fungal organisms, members of genus *Phytium* and *Lagenidium* are currently included within the class Oomycetes, and are more closely related to algae than fungi (Grooters, 2003). In the present case, the microscopical characteristics of the hyphae, together with the fact that they stained with HE, PAS and GMS, strongly suggested the diagnosis of zygomycosis (Grooters, 2003; Chayakulkeeree *et al.*, 2006). Unfortunately, specimens were not collected for culture because the lesions were presumed to be neoplastic at the time of gross examination.

Fungal or oomycetal infections in the gastrointestinal tract are described uncommonly in cats and only a few cases of intestinal phycomycosis have been reported in this species (Ader, 1979; Ossent, 1987; Rakich *et al.*, 2005; Cunha *et al.*, 2011). It is generally agreed that a heavy fungal challenge, disruption of the normal flora, a primary local lesion or lowered host resistance is required for the establishment

of mycotic disease in the gut (Grooters, 2003; Brown *et al.*, 2007). In the present case, it was not possible to determine whether the observed phycomycetes were primary or secondary to the intestinal lesion. However, it is known that the animal showed an acute clinical presentation and received no previous treatments. Tests for feline immunodeficiency virus and feline leukaemia virus were not performed, although no clinical or pathological abnormalities suggestive of these diseases were observed. Moreover, rod-shaped gram-positive and gram-negative bacteria were also observed within the lesions. The fact that they were mainly located within the area of mucosal ulceration suggests that they might have represented a secondary infection, but a potential role in triggering the observed FGESF cannot totally be ruled out.

The pathogenesis of FGESF is not understood. Craig *et al.* (2009) suggested that certain bacteria might trigger the development of an abnormal eosinophilic inflammatory response. Similar lesions found in the subcutis and abdomen of cats in Japan were proposed to be caused by meticillin-resistant *Staphylococcus* spp. (Ozaki *et al.*, 2003). However, bacterial agents are not observed in approximately 50% of cases of FGESF, and antibiotic treatments are apparently not effective (Craig *et al.*, 2009; Suzuki *et al.*, 2013). Moreover, parasites and phycomycetes typically elicit an eosinophilic reaction, which is one of the hallmarks of this condition (Grooters, 2003; Craig *et al.*, 2009; Eckstrand *et al.*, 2013). Interestingly, the most common location of gastrointestinal lesions in cases of oomycosis are the gastric outflow area (including proximal duodenum) and the ileocolic junction and the same areas are most often the site of FGESF (Grooters, 2003). However, fungal organisms have never been identified previously in these lesions.

It has been suggested that cats that develop eosinophilic reactions have an inherited eosinophil dysregulation leading to an inappropriate eosinophilic

inflammatory response to a variety of stimuli (Bloom, 2006). Thus, FGESF might represent a unique stereotypic pattern of inflammation and repair that occurs in some felids in response to tissue injury. This damage might potentially be caused by different aetiological factors including bacteria, fungi, parasites and/or trauma such as foreign body penetration (Eckstrand *et al.*, 2013) and the present case provides evidence for a role for fungal infection in some circumstances.

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Figures



Fig. 1. Stomach and duodenum. The walls of the pyloric antrum and proximal duodenum are markedly thickened and show an irregular and ulcerated mucosa.



Fig. 2. Duodenal mass. Abundant haphazardly oriented trabecular bands of dense eosinophilic and homogenous collagen containing a sparse number of large spindleshaped cells and separated by numerous eosinophils. HE.



Fig. 3. Duodenal mass. Abundant eosinophils are admixed with few, irregular, branched hyphae (arrow) and collagenous trabeculae. HE.

Fig. 4. Duodenal mass. Numerous pleomorphic, non-parallel, thin-walled hyphae are present within the collagenous trabecular bands. PAS.