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Granulomatous colitis: more than a canine disease? A case of *Escherichia coli*-associated granulomatous colitis in an adult cat

Rodolfo Oliveira Leal[1]*, Kenny Simpson[2], Mélanie Fine[3], Jean-Charles Husson[3] and Juan Hernandez[1†]

Abstract

Case summary This report describes a 4-year-old cat with chronic intermittent haematochezia and faecal incontinence of 7 months' duration. Investigation revealed severe colonic multifocal mucosal ulcerations and infiltration of the mucosal lamina propria by large numbers of periodic acid–Schiff-positive macrophages. Fluorescence in situ hybridisation analysis of colonic biopsies revealed multifocal clusters of intracellular *Escherichia coli*. Treatment with fluoroquinolones for 6 weeks led to a complete resolution of clinical signs.

Relevance and novel information The findings reveal that mucosally invasive *E coli* can also be associated with granulomatous colitis in cats and indicate the need for diagnostic testing of mucosal samples for *E coli* and other infectious agents.

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Introduction

First reported in 1965, granulomatous colitis (GC) is an uncommon form of inflammatory bowel disease (IBD) predominantly diagnosed in young Boxers and French Bulldogs.[1–4] Dogs with GC show clinical signs of colitis, haematochezia and weight loss, which can be associated with anaemia, hypoproteinaemia, cachexia and euthanasia in severe cases.[3–6] In severe cases, thickening of the colonic mucosa and ulceration are readily visualised on endoscopy, and sonography may reveal colonic thickening and regional lymphadenonathy. Histologically, GC of Boxers and French Bulldogs is characterised by infiltration of the lamina propria and submucosal layers by lymphocytes, granulocytes and macrophages containing periodic acid–Schiff-positive (PAS+) material. These cells are also found within, and surrounding lymphatics of, the tunica muscularis and serosal surface. The caecum and ileum are also involved with similar lesions, although often to a lesser degree.[3,4,6–11]

For many years, PAS+ GC in dogs was considered a severe and incurable idiopathic immune-mediated disease. The application of culture-independent methodologies to detect bacteria in fixed colonic biopsies from Boxers with GC led to the identification of multifocal clusters of mucosally invasive *Escherichia coli* within macrophages

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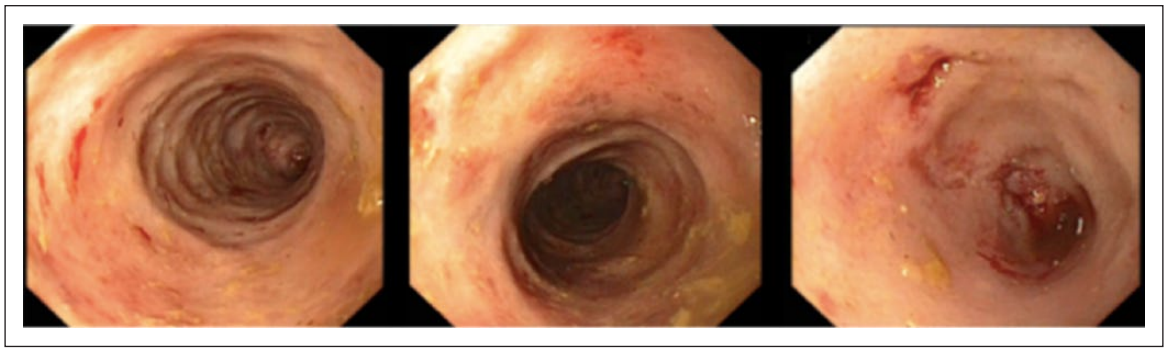

Figure 1 Colonoscopy showed thick irregular mucosa with multiple superficial ulcers (first image 11 o'clock). The mucosa was friable and bled easily during the procedure

using immunocytochemistry and fluorescence in situ hybridisation (FISH).[1,3,4] FISH analysis uses fluorescently labelled oligonucleotide probes that hybridise to bacterial 16S or 23S ribosomal DNA to localise metabolically active bacteria within formalin-fixed tissues.[1,4] FISH utilising *E coli/Shigella species*-specific probes has become the definitive test for identifying mucosally invasive *E coli* in Boxers and French Bulldogs with GC. Clinical remission and cure of GC-affected dogs correlates with the eradication of mucosally invasive *E coli* by antibiotics that are capable of penetrating macrophages and killing intracellular *E coli*. Recently, genetic analysis of affected dogs has implicated a region on chromosome 38 that is involved in detection and killing of *E coli* in other species.[12] Thus, it is emerging that *E coli*-associated GC in Boxers and French Bulldogs is likely a heritable genetic defect in sensing or killing intracellular *E coli*.

In cats, only one case of GC has been previously been reported, which was published in 1979. It describes a 5-year-old Persian crossbred cat with clinical signs of colitis. Light and electronic microscopy of colonic biopsies showed mucosal colitis with characteristic PAS+ macrophages.[13] This cat responded to chloramphenicol therapy and was healthy for at least 4 years after diagnosis. Even if at that time there was no identification of the underlying cause, the case report suggests involvement of an undiagnosed bacterial infection. It is against this background that we sought to determine the presence of mucosally invasive bacteria, including *E coli*, and clinical response to antimicrobial therapy that is effective in dogs in a cat with PAS+ GC.

Case description

A 4-year-old domestic shorthair male neutered cat was referred to the internal medicine service of Centre Hospitalier Vétérinaire Fregis (France) for chronic intermittent haematochezia and faecal incontinence of 7 months' duration. No weight loss was reported and the cat had a good appetite. Therapeutic trials with fenbendazole (50 mg/kg PO for 5 days), metronidazole (10–15 mg/kg PO q12h for 10 days) and hyperdigestible gastrointestinal diet (Hill's prescription diet i/d) were unsuccessful.

Physical examination, complete blood cell count and biochemistry panel (including serum folate and cobalamin concentration) were within normal limits.

Faecal sample for parasites by using direct smear evaluation, zinc sulfate centrifugal flotation techniques and PCR for *Tritrichomonas* and *Giardia* species were negative. Abdominal ultrasonography revealed a colonic wall thickening (2.5–3.0 mm) with attenuation of wall layering and hypo- to echoic multifocal nodules (2 mm diameter) in the submucosal layer. Colonoscopy showed an irregular and thickened colonic wall with multiple erosions, compatible with ulcerative colitis or infiltrative neoplasia (Figure 1). Colonic endoscopic biopsy samples were collected.

Biopsies were fixed in 4% saline-buffered formalin and embedded in paraffin wax. Sections of 4–5 µm were stained with haematoxylin and eosin, PAS, Toluidine blue and Fite-Faraco, and submitted for routine histopathological examination. Histopathology revealed severe multifocal mucosal ulcerations and infiltration of the mucosal lamina propria by large numbers of macrophages, with scattered small lymphocytes and plasma cells (Figure 2). The macrophages had abundant eosinophilic granular cytoplasm that was strongly PAS+. Toluidine blue and Fite-Faraco stains did not show mast cell infiltration or acid-fast bacteria, respectively. Histological findings were consistent with severe PAS+ GC similar to that documented in Boxers and French Bulldogs.

FISH analysis of colonic biopsies using eubacterial (EUB338-6FAM) and *E coli*-specific probes (*E coli*–Cy3) and a previously described technique revealed multifocal clusters of intracellular *E coli* (Figure 3).[1] More bacteria were visible with eubacterial vs *E coli* probes suggesting the possibility of a mixed infection.

Colonic swab culture was positive for *E coli* and negative for *Salmonella* species, *Yersinia* species and *Campylobacter* species. Antimicrobial susceptibility profiles of *E coli* isolates showed broad susceptibility to

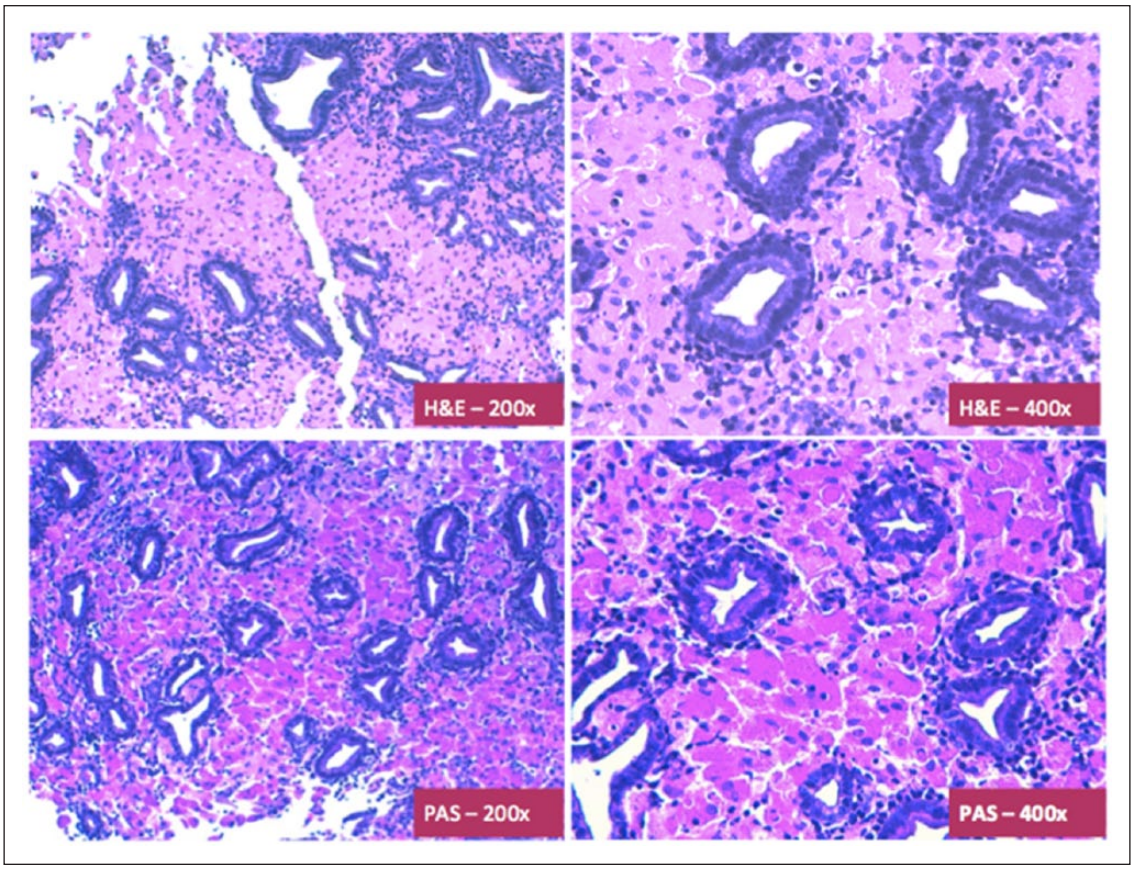

Figure 2 Histopathology of colonic biopsies showed accumulation of macrophages with abundant cytoplasm containing periodic acid–Schiff (PAS)-positive material throughout mucosal lamina propria (original photos: LAPVSO). H&E = haematoxylin and eosin

macrophage-penetrating antimicrobials (Table 1). Treatment with enrofloxacin (5 mg/kg q24h for 6 weeks) led to progressive and complete resolution of clinical signs with remission sustained for 13 months to date.

Discussion

To our knowledge, this is only the second case report of PAS+ GC in an adult cat. Historically, a primary immune-mediated pathogenesis was presumed in dogs,[5,14] but the recent identification of invasive *E coli* in dogs with GC increased our suspicion that an infectious agent could be involved and led us to perform FISH analysis, which revealed the presence of multifocal clusters of invasive intracellular *E coli*. These results reinforce the suspicion of an ineffective phagocytic activity, which can be explained either by enhanced bacterial resistance to destruction, ineffective phagocytosis or a combination of these.[13] In humans and experimental animals, it has emerged that genetic defects in bacterial sensing and killing (eg, *NOD2, ATG16L*) are frequently associated with IBD.[15–17] Variants in these genes are associated with downstream effects on protein function and, subsequently, clinical disease.[15–17] Recent studies in Boxer dogs and French Bulldogs with GC have identified a region in chromosome 38 encoding genes in the signaling lymphocyte activation molecule family that are involved in the sensing and killing of *E coli* in murine models.[12] GC in cats is extremely rare, with only two cases and two different breeds (Persian and DSH in this study) reported. It remains to be determined if a genetic basis exists.

The *E coli* strain isolated from this cat was susceptible to many different classes of antibiotics. We based antibiotic selection on susceptibility and the ability to penetrate macrophages. Owing to their lipophilicity, fluoroquinolones attain high intracellular concentrations and are effective against susceptible *E coli* within macrophages in Boxers and French Bulldogs with GC.[18] Therefore, fluoroquinolone therapy was selected. Even if several fluoroquinolones (eg, pradofloxacin or marbofloxacin) would have allowed safer use at higher concentrations, enrofloxacin was chosen as it is overall better documented in the therapeutic management of GC in dogs. However, we were aware that high doses of enrofloxacin are associated with acute retinal degeneration in cats,[19,20] although adverse effects occur generally above

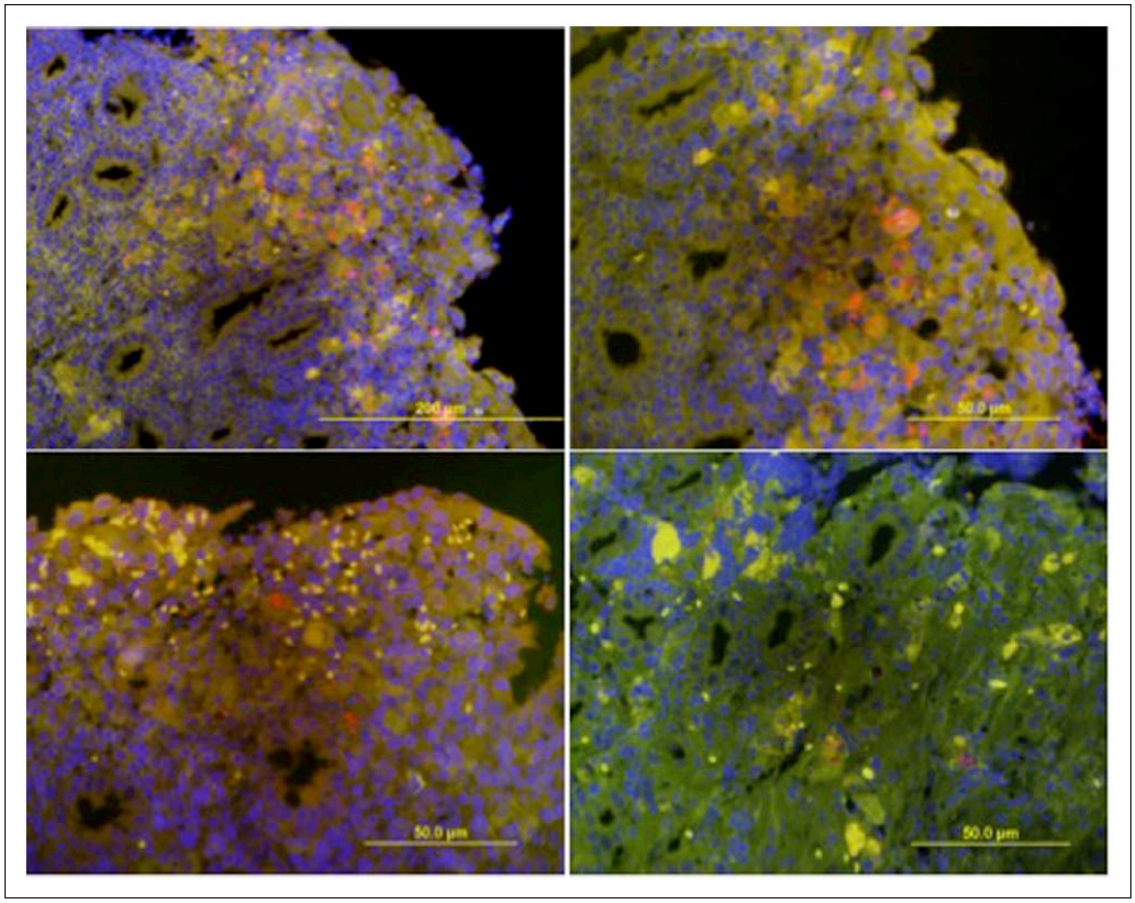

Figure 3 Fluorescence in situ hybridisation of colonic biopsies showing multifocal clusters of invasive intracellular rods (EUB-338, upper row) that hybridised with a probe to *Escherichia coli*/*Shigella species* (lower row), similar to granulomatous colitis in dogs. Bacteria stain red (cy-3). Nuclei/DNA stain blue (4',6-diamidino-2-phenylindole)

Table 1 Bacterial culture results (from colonic mucosal swab)

Aerobic culture: *Escherichia coli* +++		
Antibiotic sensitivity test results		
Beta-lactams	Potentiated amoxicillin	Sensitive
	Ampicillin	Sensitive
	Ceftiofur	Sensitive
	Cephalexin	Sensitive
	Cefovecin	Sensitive
Aminoglycosides	Gentamicin	Sensitive
	Tobramycin	Sensitive
Fluoroquinolones	Enrofloxacin*	Sensitive
	Marbofloxacin*	Sensitive
Other antibiotics	Chloramphenicol*	Sensitive
	Trimethoprim sulfonamides*	Sensitive
	Tetracyclines	Sensitive

*Antibiotics with ability to penetrate macrophages

the current dosage of 5 mg/kg/day (dose administered in this case). We chose to administer for 6 weeks as short-term duration of therapy can be ineffective in dogs with GC and is thought to relate to the time taken for mucosal healing to prevent invasion of resident *E coli*.[1,4,21] The clinical improvement observed and the complete

resolution of clinical signs with antibiotics closely paralleled findings in dog and support the direct causal role for *E coli* in feline GC.[1,4,21]

This case report had several limitations. There was no culture of colonic biopsy and only a colonic wall swab culture was performed. Although it would be interesting to have a direct colonic culture, from our point of view the rectal wall culture seemed representative of the microbial flora involved.

A second limitation was the lack of clinical work-up performed to exclude fungal infection. However, fungal diseases are extremely rare in France. In addition, PAS staining was negative for fungal organism and clinical response was complete with enrofloxacin.

A third limitation was the lack of histopathological follow-up. In dogs, a study documented that the results of PAS staining remained positive for more than 6 months, despite clinical remission.[4,21] In cats, repeat histopathology evaluation after medical therapy has not been performed. Despite long-term clinical remission confirming the success of medical therapy, it would have been informative to repeat colonic biopsies, histopathology and FISH at least 6 months after the diagnosis to determine if clinical remission was associated with eradication of intracellular *E coli* and resolution of PAS+ colitis. However, in the face of successful medical therapy and long-term remission, a repeat biopsy was considered optional rather than essential.

Conclusions

This case report revealed that *E coli*-associated GC can also affect cats and should be considered in the differential diagnosis of chronic haematochezia in this species. Further studies are needed to assess molecular, genetic and immune pathways underlying intracellular invasion by *E coli* in cats.

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