Spontaneously arising disease

A case of canine intestinal malakoplakia

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ABSTRACT

Malakoplakia is a rare chronic granulomatous disease usually affecting the urinary bladder and other locations. In humans, the gastrointestinal tract is the second most common location but there are no reports of intestinal malakoplakia in animals. A 10-month-old female French Bulldog was presented with chronic haemorrhagic diarrhoea and anorexia with normochromic-normocytic anaemia and hypoalbuminaemia. Grossly, there was mucosal thickening and ulceration of the caecum, colon and rectum. Microscopically, transmural sheets of foamy macrophages were seen in these tissues. Macrophages were periodic acid—Schiff, vimentin and ionized calcium-binding adaptor molecule 1 positive and contained von Kossa- and Prussian blue-positive Michaelis—Gutmann bodies. Giemsa staining revealed rod-shaped bacterial colonies and fluorescence in-situ hybridization demonstrated *Escherichia coli* within macrophages. This is the first reported case of intestinal malakoplakia in domestic animals. Pathological features of intestinal malakoplakia share many similarities with ulcerative histiocytic colitis in dogs but it is unclear if they are different forms of the same pathological process or distinct entities.

Malakoplakia is a rare chronic granulomatous disease of humans [1,2] and several animal species including pigs, cats, dogs and macaques [3—11]. In domestic animals, this disease commonly affects the urinary tract, especially the urinary bladder [1,9], although it can be found in other locations such as the kidneys [11], pancreas, stomach and omentum [5], vagina [7] and lymph nodes [3] and may also occur systemically [4]. In humans, malakoplakia is associated with immunosuppressive states [1,12], whereas it appears to arise spontaneously in animals. The pathogenesis is mainly related to *Escherichia coli* infection [6—9], together with a defect in the capacity of macrophages to digest bacteria [13]. In these cases, there are deficiencies in phagolysosome structure and function [14—16] possibly associated with inefficient assembly of microtubules, which is essential for the invagination and degranulation of bacteria [12]. Macroscopic lesions in humans have been described as generalized nodules, polyps [2] or a diffuse thickening [17]. Histologically, malakoplakia is characterized by sheets of activated macrophages with numerous intracytoplasmic periodic acid—Schiff (PAS)-positive granules, the so-called von Hansemann-type macrophages [9,12].

Ultrastructural examination has identified these intracytoplasmic granules as lysosomes and phagolysosomes, occasionally filled with bacteria and bacterial debris. The diagnosis of malakoplakia is based on the identification of Michaelis—Gutmann (MG) bodies [1,12], lamellar inclusions with various degrees of mineralization, in the cytoplasm of macrophages and in extracellular locations [9]. MG bodies are formed by bacterial detritus and mineral deposits, mainly iron and calcium and, to a lesser extent, phosphorus, chloride and sulphur [14,15].

In humans, the gastrointestinal tract is the second most common location affected by this disease [1]. However, intestinal malakoplakia has not been reported in domestic animals [9]. We now document the first case of intestinal malakoplakia in any animal species.

A 10-month-old female French Bulldog was presented with a history of chronic haemorrhagic diarrhoea and anorexia. Haematological and biochemical tests revealed normochromic and normocytic anaemia (haematocrit 24—35% [reference interval (RI) 37.3—61.7] and haemoglobin 12.3 g/dl [RI 13.1—20.5]), together with hypoalbuminaemia (1.8 g/dl [RI 2.3—4.0]). Ultrasound images revealed marked thickening of the colonic and caecal walls and enlarged mesenteric lymph nodes. The animal was euthanized and referred for post-mortem examination.

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Tissue samples obtained at necropsy were fixed in 10% neutral-buffered formalin and embedded in paraffin, and 4-μm thick sections were stained with haematoxylin and eosin (HE), PAS, von Kossa, Prussian blue, Gram, Giemsa and Ziehl—Neelsen (ZN) stains. Immunohistochemical labelling was carried out to characterize the cellular composition of the lesions using antibodies against ionized calcium-binding adaptor molecule 1 (Iba1), vimentin and pancytokeratins (panCKs). For Iba1, tissue sections were incubated with anti-Iba1 primary antibody (1:1000; clone 013-27691; FUJI-FILM Wako Pure Chemical Corporation, https://labchem-wako.fujifilm.com) at room temperature for 30 min. Heat-induced epitope retrieval (HIER) with Tris/EDTA at pH 9 was used and sections were labelled with EnVision DAB (Dako, www.agilent.com). For vimentin, sections were incubated with monoclonal mouse anti-vimentin antibody (ready-to-use; clone V9; Agilent Dako) at room temperature for 20 min. HIER was carried out with citrate buffer at pH 6.1 and sections were labelled with EnVision DAB. For panCKs, sections were incubated with monoclonal mouse anti-human cytokeratin antibody (ready-to-use; clone AE1/AE3; Agilent Dako) at room temperature for 60 min. HIER was carried out as for vimentin and sections labelled with EnVision DAB. Samples of a canine cutaneous histiocytoma, normal colon and epidermis were used as tissue-positive controls for Iba1, vimentin and panCKs, respectively. Sections of normal canine skin and large intestine were used as negative controls.

Grossly, the mucosa of the distal colon and rectum was multifocally thickened by nodular masses (2.5 cm) and occasionally ulcerated (Fig. 1). The caecal mucosa was diffusely thickened. On sectioning, both thickenings had a whitish and homogeneous appearance and hard consistency. Mesenteric lymph nodes were enlarged. Microscopically, the distal colon, rectum and caecum were transmurally thickened by sheets of activated macrophages admixed with a few lymphocytes, plasma cells and neutrophils (Fig. 2). Multifocally, the mucosa was ulcerated and replaced by a similar granulomatous infiltrate. Macrophages were round to polygonal, with abundant cytoplasm containing eosinophilic granular inclusions (von Hansemann-type macrophages) that frequently displaced the nucleus peripherally. Macrophages invaded the capsule and the subcapsular sinus of mesenteric lymph nodes. Macrophages stained intensely with PAS (Supplementary Fig. 1) and were immunopositive for vimentin (Supplementary Fig. 2) and Iba1 (Supplementary Fig. 3). Multiple intracytoplasmic calcified spheroidal bodies, compatible with MG bodies, were seen. Depending on the mineral organization, the MG bodies varied from eosinophilic to basophilic (Fig. 3) and were positive with von Kossa (Supplementary Fig. 4) and Prussian blue stains (Supplementary Fig. 5). Colonies of rod-shaped bacteria within the cytoplasm of macrophages, and also located extracellularly, were Giemsa positive (Fig. 4) and Gram and ZN negative.

Samples of the affected colon were stored at −80°C and tested using real-time polymerase chain reaction (RT-PCR) (Exopol, www.exopol.com) against different canine intestinal-associated viral pathogens (canine distemper virus, canine parainfluenza, canine adenovirus types I and II, canine parvovirus and canine herpesvirus I). Fluorescence in-situ hybridization (FISH) was performed (IDEXX Laboratories, www.iddex.es) to detect intracytoplasmic *E. coli* within macrophages. RT-PCR for canine distemper virus, canine parainfluenza, canine adenovirus types I and II, canine parvovirus and canine herpesvirus I was negative. FISH revealed numerous positive signals in the cytoplasm of macrophages compatible with *E. coli*. The diagnosis of malakoplakia was made on the basis of the results of histopathology, histochemistry and immunohistochemistry against Iba1 and vimentin on sections of the distal colon, rectum, caecum and mesenteric lymph nodes.

Fig. 1. Malakoplakia, rectum, French Bulldog. Transmural thickening (arrow) with multifocal ulcers. Defined nodule also present (arrowhead). Bar, 1 cm.
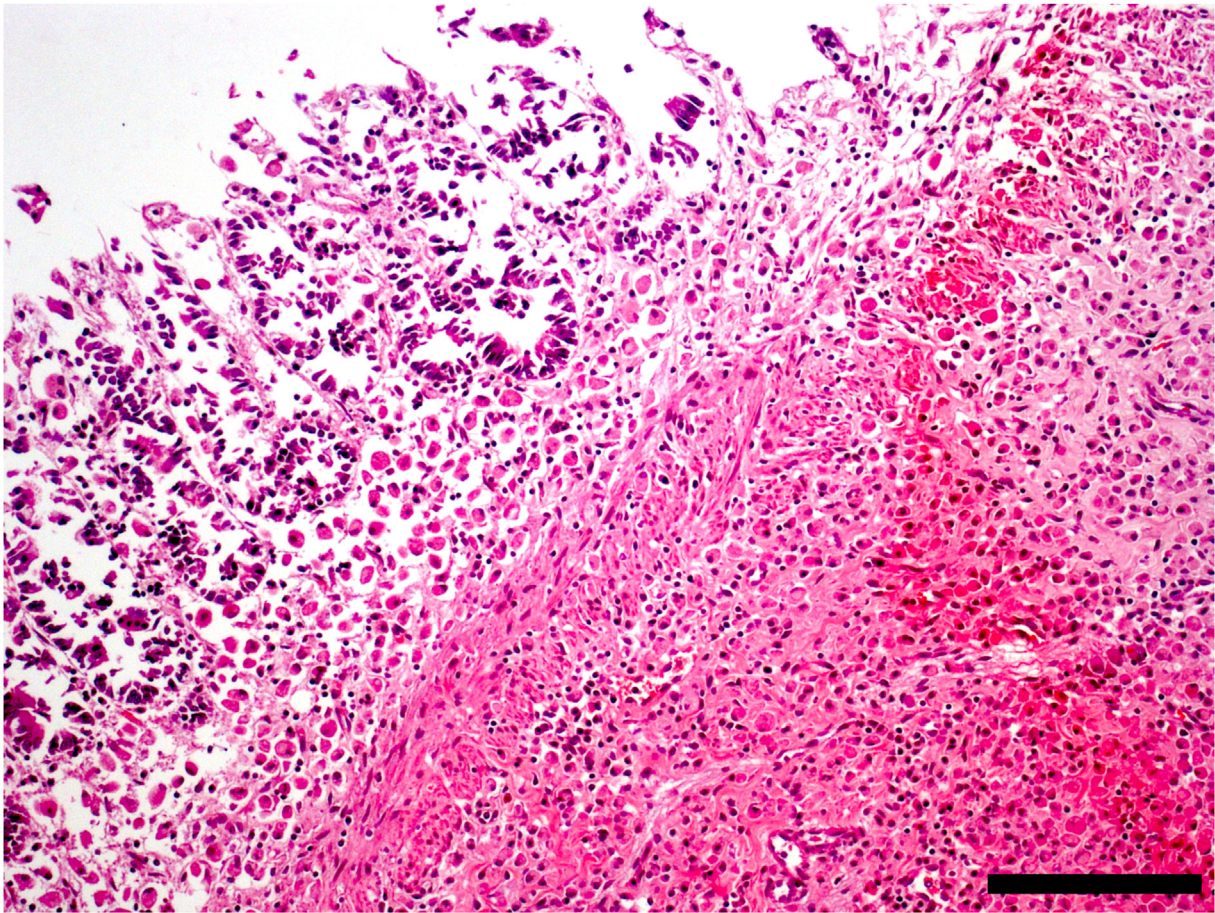
Fig. 2. Malakoplakia, distal colon, French Bulldog. Severe, diffuse, von Hansemann-type macrophage infiltrate, effacing colonic architecture. HE. Bar, 300 μm.
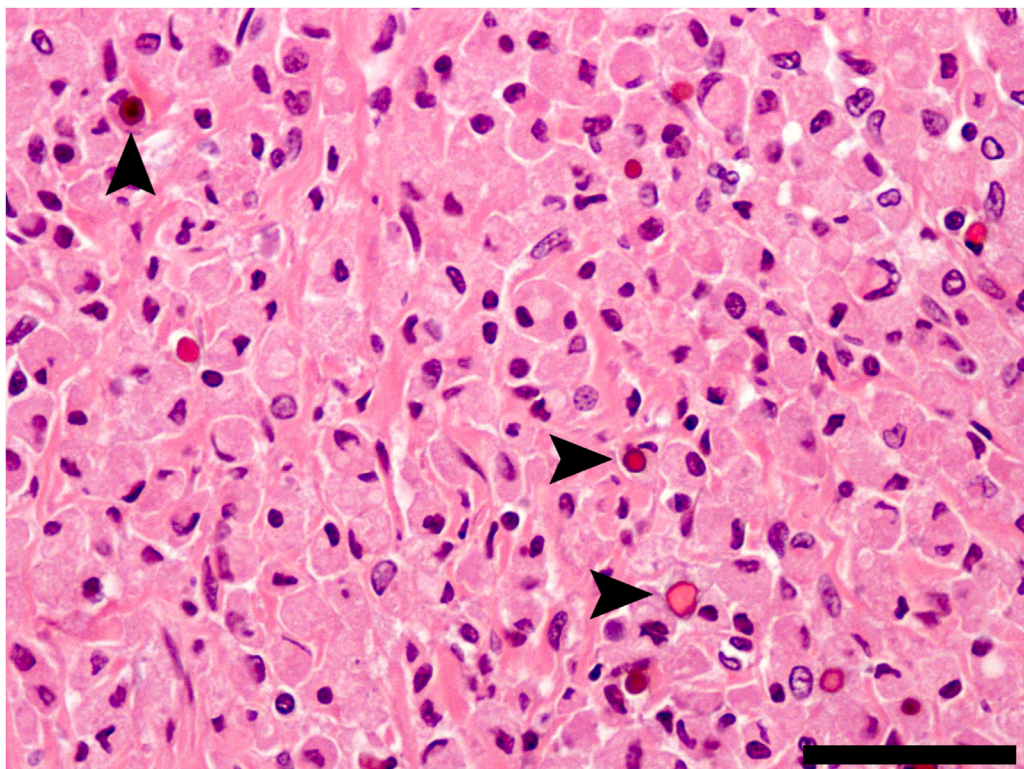

Fig. 3. Malakoplakia, distal colon, French Bulldog. Michaelis−Gutmann bodies in cytoplasm of macrophages (arrowheads) have variable degrees of mineralization. HE. Bar, 100 μm.
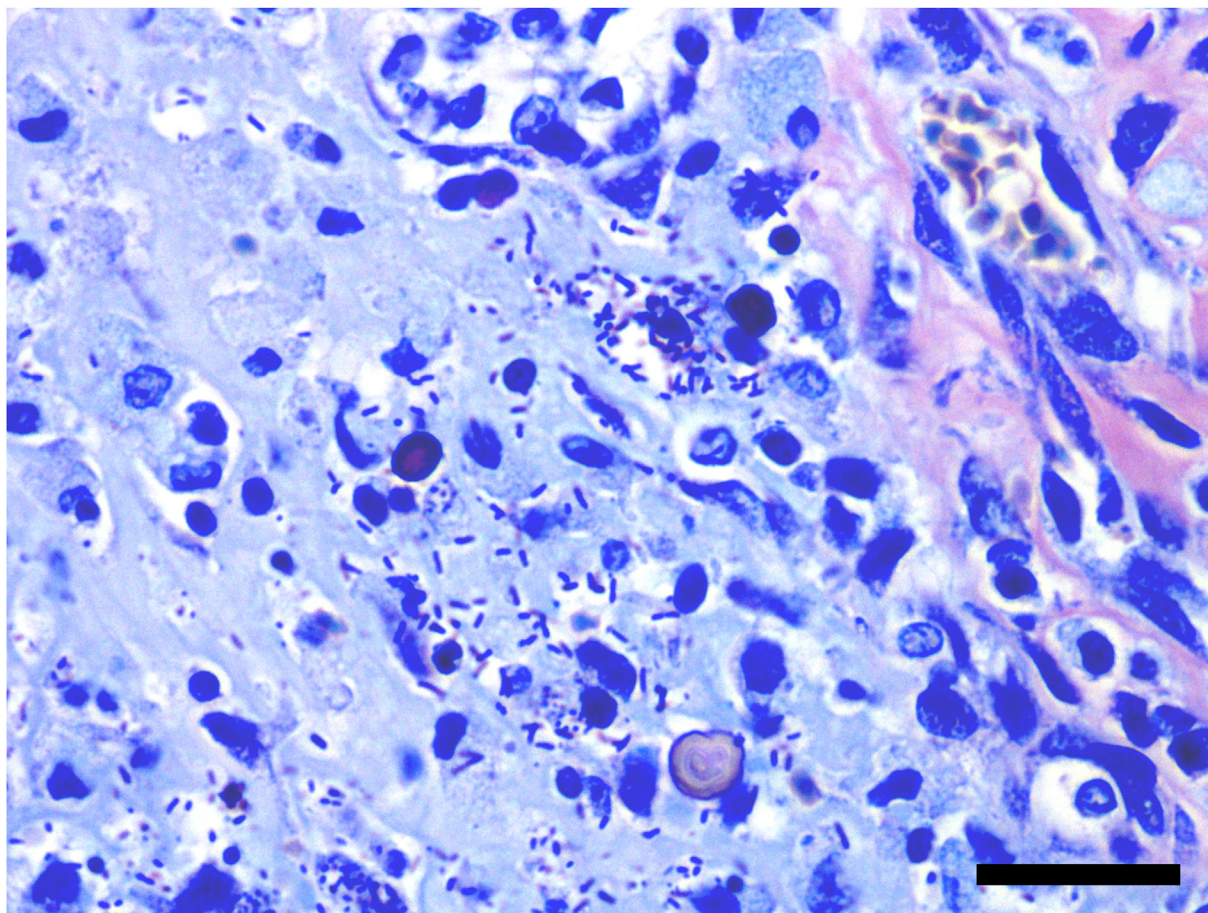
Fig. 4. Malakoplakia, distal colon, French Bulldog. Extra- and intracellular coccobacilli in von Hansemann-type macrophages. Giemsa. Bar, 100 μm.

To the best of our knowledge, this is the first case of intestinal malakoplakia reported in domestic animals. Malakoplakia is a chronic granulomatous disease linked to an inherent defect in macrophage bacterial digestion [13]. The cytoplasm of macrophages is distended by numerous lysosomes and phagolysosomes [9,13—15]. However, macrophages have reduced guanosine 3′,5′-cyclic monophosphate/adenosine 3′,5′-cyclic monophosphate and abnormal β-glucuronidase activities, indicating a deficiency in their phagolysosomal activity [15]. In domestic animals, a chronic bacterial infection (most commonly *E. coli*) has been proposed as the aetiology of this condition [6—9], although other bacteria such as *Mycobacterium avium* [3] and experimental infection by *Rhodococcus equi* in swine [16] have been implicated. The proposed pathogenesis of malakoplakia is phagocytosis of bacteria by macrophages with defective phagolysosomal activity (pre-phagosomal stage) resulting in partial digestion (phagosomal stage), leading to accumulation of glycolipid residues in the cytoplasm. These glycolipids could act as nucleation centres for subsequent calcium and iron deposition (post-phagosomal stage) [13]. The main feature of malakoplakia is the presence of MG bodies, which appear as lamellar inclusions, are only observed in the final phase of the disease (post-phagosomal stage) and are identified principally by von Kossa staining [1,9,12]. In the reported animal cases of malakoplakia, Prussian blue staining produced inconsistent results [3,4,9]. In this case, the vast majority of MG bodies were von Kossa

and Prussian blue positive. Variability in von Kossa and Prussian blue positivity depends on the extent of calcium/phosphorus/iron deposition [12].

In domestic animals, malakoplakia mainly affects the urinary system [9]. Our results indicate that the pathological features of intestinal malakoplakia are closely related to granulomatous colitis [18], also called ulcerative histiocytic colitis (UHC), in Boxers and Bulldogs [19]. Both diseases have been described in young animals from 4 to 42 months of age. In the present case, malakoplakia affected the distal colon, caecum and mesenteric lymph nodes, which are the anatomical locations affected by UHC. Microscopically, PAS-positive macrophages are characteristic of both of these conditions [18,19] but in domestic animals malakoplakia is exclusively diagnosed by the presence of MG bodies. As these inclusions are only seen in the final phase of malakoplakia, the early stages of this disease may be impossible to differentiate from UHC. Both diseases are mainly caused by chronic *E. coli* infection [18,19] and can be treated with enrofloxacin [7,19]. In Boxers and Bulldogs a polymorphism on chromosome 38, a specific region that includes members of the signalling lymphocytic activation molecule (SLAM) family, has been associated with the development of both diseases [20]. A deficiency in phagocyte SLAMF1 can induce an altered macrophage response to *E. coli* infection, leading to a state of macrophage hyperactivation in the lamina propria and dysregulation of inflammatory cytokines [21].

In conclusion, we document the first case of intestinal malakoplakia in an animal species. Intestinal malakoplakia in this dog shares many similarities with UHC but it is unclear if they represent different forms of the same pathological process or are distinct entities.

Statement of author contributions

Á. Gómez: Conceptualization, Methodology, Investigation, Resources, Visualization, Writing — original draft, review and editing. **E. Pérez:** Investigation, Resources. **N. Calvo-Sánchez:** Investigation, Resources. **M. Pérez**: Investigation, Resources. **M. Borobia:** Methodology. **L. Luján:** Investigation, Resources, Writing — review and editing, Supervision. **A. Rodríguez-Largo:** Investigation, Resources, Writing — review and editing, Supervision. **S. Villanueva-Saz:** Investigation, Resources, Writing — review and editing, Supervision.

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Declaration of competing interests

The authors declared no conflicts of interest in relation to the research, authorship or publication of this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcpa.2023.07.002.

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