



CASE REPORT

An Unusual Case of Megacolon Due to *Sarcocystis* spp. Infection and Local Amyloidosis in a Husky Dog

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ABSTRACT

A four-year old Husky dog suffering of chronic diarrhea was radiologically diagnosed with megacolon and consequently, partial resection of the colon wall was performed. Histological examination of the colonic wall revealed the presence of *Sarcocystis* spp. oocysts. Chronic inflammation of colonic mucosa resulted in local amyloidosis, overexpression of Bcl-2 in the goblet cells, which suggested decreased apoptosis and increased mucous cells population in Lieberkühn's glands. The expression of immunoglobulin A (IgA) was reduced intra-epithelially and increased in blood capillaries, periglandular and in the submucosal stroma consequently to parasitic infection. The colonic mucosa showed plasma cells, eosinophilic, mast cells and lymphocytic infiltrations. The *Sarcocystis* chronic parasitism may cause colon chronic dilatation due to persistent inflammation of the mucosa, characterized by infiltration of lymphocytes and plasma cells followed by the synthesis of amyloid. The amyloid stored around the Lieberkühn's glands or in the submucosal stroma can cause autonomic dysfunction in Auerbach's and Meissner's plexuses and autonomic ganglia.

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INTRODUCTION

The megacolon is an abnormal dilatation of the colon. The colon is responsible of both ion and water absorption and has a significantly reduced role by itself in nutrient digestion (Bharucha and Phillips, 1999). The colon of dogs and cats is relatively short, measuring 10-20% of the intestinal length. The megacolon can be acute, chronic or toxic. The chronic form can be congenital, primarily or secondarily acquired and idiopathic (Meier-Ruge *et al.*, 2006). The idiopathic megacolon pathogenesis is controversial.

Parasitic etiology of megacolon was also reported in humans. In Central and South America, the most common incidence of the chronic megacolon was observed in approximately 20% of human patients suffering from Chagas disease caused by *Trypanosoma cruzi* (Bharucha and Phillips, 1999).

History and clinical examination: A four-year Husky dog with chronic diarrhea was examined. The case history

consisted in watery diarrhea alternating with little more consistent feces embedded in mucus, enhanced appetite and weight loss. antiparasitic substances - drontal (praziquantel 50 mg + pyrantel embonate 144 mg + febantel 150 mg; Bayer Animal Health GmbH, Germany) and Cestal (praziquantel 50 mg + pyrantel embonate 144 mg + febantel 150 mg; Ceva Sante Animale Romania SRL, Romania) and antibacterial substances - Ercefuryl (nifuroxazide 200 mg; Sanofi-Aventis, France) had been previously administered to the patient, but no clinical improvements were observed.

Hematological (RBC, WBC, MCV, MCH, MCHC) and biochemical (ALT, AST, blood urea nitrogen - BUN and creatinine) investigations were conducted. Willis method for parasites in feces and radiological examination after barium enema were performed in order to aid the diagnosis.

The hemogram and biochemical indexes were found within physiological limits; the coproscopic examination was negative. Radiography showed the presence of megacolon (Fig. 1).

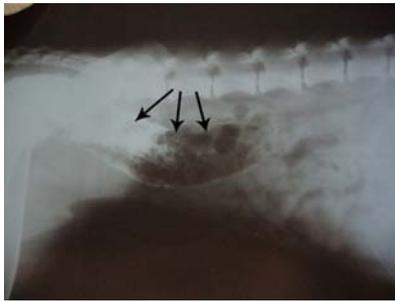


Fig. 1: Megacolon (arrows). Radiological image after barium enema

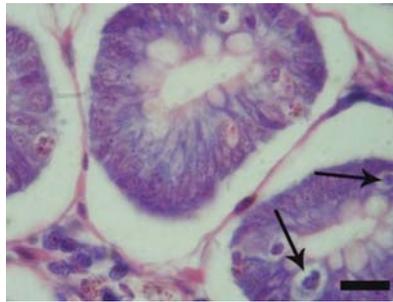


Fig. 2: Intraepithelial *Sarcocystis* spp. oocysts and macrogametes (arrows). Plasma cells periglandular infiltration. Giemsa stain. Bar=20 µm

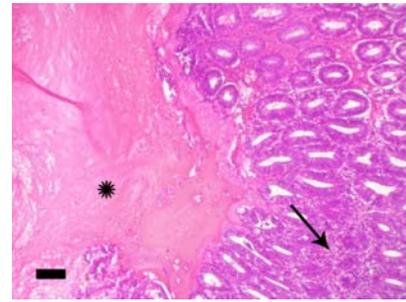


Fig. 3: Amyloid in the colonic submucosa (star); *Sarcocystis* spp. oocysts (arrow). PAS stain. Bar=200 µm

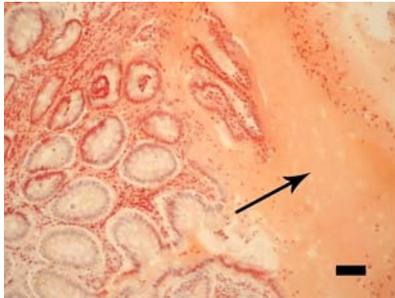


Fig. 4: Amyloid in the colonic submucosa (arrow); some glands were partially damaged Congo Red stain. Bar=200 µm

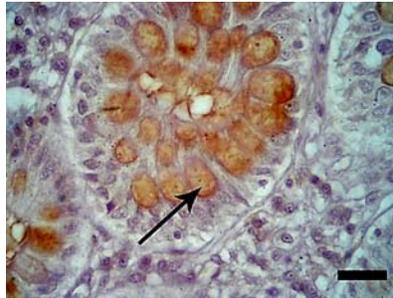


Fig. 5: Colon - Overexpression of Bcl-2 into the goblet cells (arrow). Bcl-2 IHC. Bar=20 µm

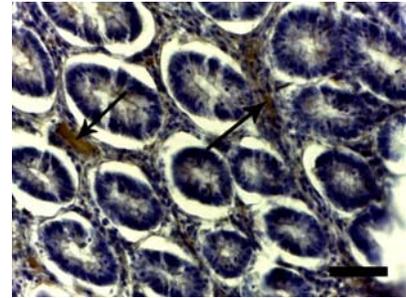


Fig. 6: Weak expression of IgA intraepithelial cells but it increased in lamina propria (arrows). IgA IHC stain. Bar=50 µm

Diagnosis and treatment: Surgical partial colon resection was conducted following the procedure described by Abedi *et al.* (2012). After surgical intervention, the patient was medicated with sulfamethoprim (sulfamethoxazole) 25mg/kg BW for seven days and was cured after a short period of convalescence. A clinical survey was performed every month, during one year after the cure. The dog found healthy and appeared to have a normal intestinal discharge.

After surgical correction of the colon wall, histological and immunohistochemical examinations were conducted. Colon pieces were fixed with 10% formaldehyde, dehydrated with ethanol and embedded in paraffin. After cutting at 5 µm, the sections were dewaxed and stained with hematoxylin eosin (HE), Congo red, Periodic Acid Schiff (PAS), Giemsa for tissues and immunohistochemically with Bcl-2 and IgA antibodies. For Bcl-2 immunohistochemistry (IHC) the sections were dewaxed and blocked epitopes in the sections were revealed by heating at 95°C in 10 mmol citrate acid buffer pH 6 for 10 minutes in a microwave; the sections were then left at room temperature for 20 min. The slides were then washed twice in PBS (pH 7.5) for 5 minutes and incubated with normal serum goat, then with the first antibody -Purified Mouse Anti human Bcl-2 (Pharmigen Biosciences, Cat No 610539) diluted 1:50 at room temperature in humid chamber for one hour. After washing with PBS, slides were incubated with the secondary antibody, HRP Goat anti Mouse IgG (BD Biosciences Pharmigen Cat.554002) for 1 hour in humid chamber at 4°C, then washed with PBS and incubated with the DAB Substrate (BD Biosciences Pharmigen; cat no. 550880) for 5 min and counter-stained with Harris hematoxylin and clarified in xylene. Slides were then mounted and examined by transmission microscopy. For IgA IHC primary antibody IgA diluted

1:100 overnight in humid chamber at 4°C, blocking serum (horse serum) and biotinylated universal secondary antibody, streptavidin HRP (NVD Leica) and DAB were used; the slides were counter-stained with Harris hematoxylin and clarified in xylene.

The histological examination of the colonic wall revealed intermediary forms of *Sarcocystis* spp. oocysts (Fig. 2). Histological examination of the colon wall revealed the presence of both *Sarcocystis* spp. (Fig. 3) and the amyloid deposits at the bottom of the Lieberkühn's glands, sometimes within them; some of glands were partially damaged. Amyloid material was also seen in the submucosal stroma. The amyloid had a pink color under PAS staining (Fig. 4), orange color under Congo Red (Fig. 5) staining and a green color under polarized light.

Edema was observed around the glands and also lymphoid infiltration, small quantities of amyloid secretion, large numbers of plasma cells, eosinophils and mast cells. In our case amyloid deposits were associated with plasmacytosis. Various intermediary forms of *Sarcocystis* spp. were found in the colonic epithelial cells.

The Bcl-2 staining revealed positive goblet cells, many of which were hypertrophic (Fig. 5). A reduced expression of IgA in intraepithelial cells and an increased one in lamina propria and in submucosal stroma was also observed (Fig. 6). Cross-sections of Lieberkühn's glands from the dog patient contained 15 goblet cells in comparison with 8/section in healthy animals.

DISCUSSION

The genus *Sarcocystis* includes over 130 species, but the taxonomic issues are still discussed. They are generally diheteroxenous sporozoa whose definitive host is a

carnivore or omnivore mammal. The definitive hosts eliminate the sporocysts through feces, each containing four sporozoites. In the intestines, the sporozoites leave the sporocysts and invade the intestinal mucosa. Dog is the definitive host for several species of intestinal *Sarcocystis*: *S. cruzi*, *S. capracanis*, *S. hircicanis*, *S. fayeri*, *S. meischeriana*, but could be also an intermediate host for some extra-intestinal species like *S. canis*, whose definitive host remains still unknown (Brömel and Sykes, 2005).

In our case chronic parasitosis caused a localized amyloidosis of colonic mucosa, as a consequence of predominantly plasma-cells infiltration. Amyloidosis can have a systemic development with numerous deposits in various tissues/organs or a local occurrence affecting a single tissue/organ. The former can be a primary amyloidosis which is accompanied by immunocytes unbalance or a secondary one associated with chronic inflammatory processes or tissue damages. The secondary amyloidosis associated with chronic inflammations is the most common form in animals (Leung *et al.*, 2012). Reactive amyloidosis was reported in some canine breeds (a chronic inflammatory disease associated with tissue deposits of amyloid A proteins). In reactive amyloidosis the amyloidic proteins form extracellular deposits which shroud the cells and block their metabolism.

The amyloid deposits can be encountered in a limited zone of the body as localized amyloidosis. Various forms of amyloid deposits are reported in both man and animals (Leung *et al.*, 2012). In man, pathophysiology of amyloid induced chronic diarrhea is associated with autonomic dysfunction due to amyloid depositions in Auerbach's and Meissner's plexuses and autonomic ganglia (Fonnesu *et al.*, 2009). A similar pathogenic mechanism may be involved in our case.

Primary amyloidosis is generally caused by clonal expansion of plasma cells which produce Ig light chains which subsequently deposit as amyloid fibrils in tissues. Amyloidosis is the effect of the cell/tissue invasion by the insoluble amorphous eosinophilic hyaline proteic deposits called hyaline (Cywinska *et al.*, 2010).

Amyloidosis is very rare in animals. Local deposit of amyloid was reported in different species as nodular tracheal/bronchial form in dogs, cutaneous nodular amyloidosis in horses and colonic amyloidosis in baboons.

Previous reports on amyloid A documented the resorption of amyloid when the stimulus for amyloid formation was eliminated. A suitable hypothesis would be that the disappearance of amyloidogenic components (e.g. amyloid A, light chains) could restore cellular regeneration despite the local presence of amyloid mass (Zeier *et al.*, 2003).

The goblet cell hyperplasia in colonic mucosa, with Bcl-2 positive secretion was observed. This may be associated with a Th-2 immune response which is mainly controlled by IL-13 and also IL-4 in parasitic infections (Marillier *et al.*, 2008). Intreepithelial IgA expression, revealed by IHC, was reduced in our case, but increased periglandularly, into the capillaries and in submucosal stroma. Gastrointestinal goblet cells are responsible for producing and maintaining a protecting mucous layer formed by the synthesis and secretion of glycoproteins known as mucoproteins/mucins. Mucins are susceptible to be the first molecules to interact with the invading pathogens on the cell surface and consequently they can limit the pathogen bondage to other glycoproteins and thus neutralize their action (Kim and Khan, 2013).

Bcl-2 is an inhibitor of apoptosis. In our case Bcl-2 expression sustains goblet cell metaplasia in dog. Pathological stress caused by *Sarcocystis spp.* infection induces inflammation and tissue ulceration, which may lead to intestinal metaplasia.

The *Sarcocystis* chronic parasitism may cause megacolon due to persistent inflammation of the colonic mucosa, characterized by lymphoplasmacytic infiltration followed by the synthesis of the amyloid. This is stored around the Lieberkühn's glands or in the submucosal stroma can cause autonomic dysfunction in Auerbach's and Meissner's plexuses and autonomic ganglia.

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