Degenerative Liver Disease in Young Beagles with Hereditary Cobalamin Malabsorption Because of a Mutation in the Cubilin Gene

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Cobalamin is an essential cofactor for enzyme systems, and adequate amounts are required for nucleic acid synthesis and hematopoiesis.[1] The 2 most important reactions involving cobalamin are the conversion of methylmalonyl-coenzyme A to succinyl-coenzyme A and the remethylation of homocysteine. Cobalamin deficiency leads to decreased activity of these enzyme systems, resulting in increased concentrations of urinary methylmalonic acid and total plasma homocysteine. Dogs are unable to synthesize cobalamin and rely on uptake of dietary cobalamin, which is bound in the small intestine to the secreted protein, intrinsic factor (IF). The cobalamin-IF complex binds to the membrane-bound cubam receptor, which mediates endocytosis. Cubam consists of 2 separate protein subunits, amnionless (AMN) and cubilin (CUBN).[1] In infants, mutations in either the *AMN* or *CUBN* genes lead to Imerslund-Gräsbeck syndrome (IGS).[2] This is a rare autosomal recessive disorder which, if left untreated, results in failure to thrive, megaloblastic anemia, proteinuria, and neurological damage.[2] In dogs, primary cobalamin malabsorption, which is analogous to IGS in humans, has been reported in young Australian Shepherds,[3] a Beagle,[4] Border Collies,[5–7] and Giant Schnauzers.[8] The genetic defects in affected Border Collies and Beagles recently have been identified as 2 independent mutations in the *CUBN* gene.[9,10] Similar to human patients, dogs typically present at a young age with inappetence, weakness, and failure to thrive.[4–6,8,11] Although liver disease is recognized in cobalamin-deficient farm animals, especially lambs,[12,13] it has not been reported in dogs suffering from hereditary cobalamin malabsorption. The aim of the present case series was to describe the clinical and histopathologic findings of liver disease in 2 client-owned beagles suffering from genetically confirmed cobalamin malabsorption.

Genetic studies

Analogous to the recent identification of the causative mutation in cobalamin-deficient border collies,[9] a whole genome resequencing approach was used in the described beagles. Stored blood samples (EDTA) from case 1 and a paraffin-embedded formalin-fixed liver sample from case 2 were used to isolate genomic DNA. Both cases were retrospectively confirmed to carry the CUBN:c.786delC mutation in a homozygous state using the recently described genotyping method.[10]

Histopathology

Histopathology samples were examined and graded by a board-certified pathologist (MR).[a]

Case 1

A 12-month-old, intact, female Beagle was referred for anorexia and cachexia. Inappetence was first noted when the dog was 5 months old. A biochemistry profile performed by the referring veterinarian disclosed mildly increased alanine aminotransferase activity (ALT 185 IU/L; reference interval [RI], 8–75 IU/L). Serum thyroxine, canine thyroid-stimulating hormone, and canine trypsin-like immunoreactivity (cTLI) concentrations were within reference intervals. One week before presentation, a computerized tomography (CT) scan of the brain (to rule out hydrocephalus) and gastroduodenoscopy were performed at a private clinic. The CT scan images, and gastric and duodenal biopsies were considered unremarkable by a board-certified radiologist and a board-certified pathologist (MR), respectively. On presentation, the dog weighed 4.0 kg with a body condition score (BCS) of 2/9. The dog

seemed lethargic, but a neurologic examination was unremarkable. A complete blood count (CBC) and serum biochemistry profile identified marked neutropenia (1,680/µL; RI, 2,496–7,437/µL), mild hyperbilirubinemia (0.37 mg/dL; RI, 0–0.20 mg/dL), moderately decreased urea nitrogen concentration (BUN, 3.9 mg/dL; RI, 10.6–26.3 mg/dL), mild hypoproteinemia (4.7 g/dL; RI, 5.6–7.1 g/dL), and mildly increased serum alkaline phosphatase (ALP, 199 IU/L; RI, 20–98 IU/L). Results of an ammonia tolerance test and of coagulation times (PT, aPTT, thrombin time) were within reference intervals. Urinalysis disclosed mild proteinuria (urine protein-to-creatinine ratio [UPC], 0.86; RI, 0–0.3). Glucocorticoid deficiency was ruled out by appropriate results of an ACTH stimulation test. Thoracic radiographs and abdominal ultrasound examination were unremarkable with the exception of mild peritoneal effusion. Analysis of abdominal fluid was consistent with a pure transudate (total protein, <1.0 g/dL; nucleated cell count, 50/µL). Histopathologic examination of ultrasound-guided liver biopsy samples (16 G) disclosed multifocal groups of markedly swollen hepatocytes with either foamy cytoplasm and glossy-appearing nuclei or small clearly demarcated vacuoles (Fig 1). Small lipofuscin deposits were visible in some hepatocytes and Kupffer cells. Multifocal areas of single-cell necrosis and mild periportal lymphoplasmacytic infiltration were present. A modified Gömöri[b] stain identified a fine panlobular sinusoidal reticulin fiber network (Fig 2). The interpretation was marked hepatocellular degeneration with secondary mild lymphoplasmacytic hepatitis.

Because similar clinical signs were described previously in a cobalamin-deficient Beagle,[4] serum was submitted for cobalamin and folate concentrations, identifying a cobalamin concentration below the
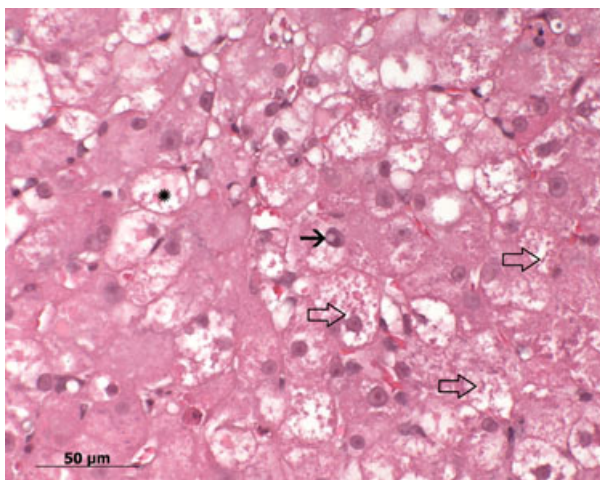

Fig 1. Photomicrograph of hematoxylin-eosin–stained liver sections of a Beagle with hereditary cobalamin malabsorption (Case 1) showing markedly swollen hepatocytes with foamy cytoplasm (glycogen storage, asterisks); the nuclei of the hepatocytes are often round and swollen with a glossy appearance secondary to glycogen deposition (slender arrow). Fine granular pigment (lipofuscin, broad open arrows) is visible in a few hepatocytes.
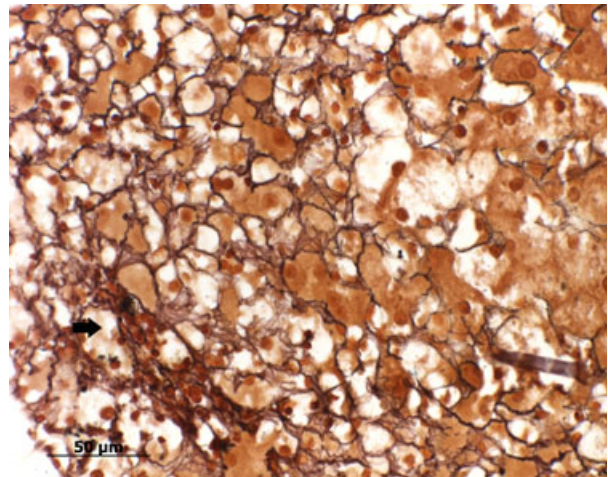

Fig 2. Photomicrograph of a Gömöri-silver–stained liver section of a dog with hereditary cobalamin malabsorption (Case 1) showing increased numbers of reticulin fibers around the central vein (broad arrow), radiating into the sinusoids of the liver lobules.

measurement limit (<150 ng/L; RI, 261–1,001 ng/L). The subsequent finding of marked methylmalonic aciduria (5,460 mmol/mol creatinine; RI, <2 mmol/mol) and hyperhomocysteinemia (69.9 µmol/L; RI, 4.3–18.4 µmol/L) was supportive of a cobalamin-depleted state.[7] The dog was treated for cobalamin deficiency with cyanocobalamin[c] (50 µg/kg IM q7d) and for the concurrent liver disease with prednisolone[d] (5 mg [1.25 mg/kg] PO q24h) and S-adenosylmethionine[e] (90 mg PO q24h) and discharged from the hospital.

The dog's clinical condition and appetite improved rapidly. On day 32, the dog weighed 5.3 kg (weight gain of 1.3 kg), was alert and active. Serum cobalamin concentration (4 days postinjection) was within reference intervals (591 ng/L; RI, 261–1,001 ng/L). On day 134, the dog weighed 6.5 kg (weight gain of 2.5 kg). A CBC and serum biochemistry profile disclosed mildly decreased BUN concentration (6.7 mg/dL; RI, 10.6–26.3 mg/dL) and mildly increased ALT (141 IU/L; RI, 20–93 IU/L). The dosage of prednisolone was tapered over 1 week and discontinued. Weekly cobalamin injections were continued. Thirty-six months after initial presentation, the owner requested re-evaluation. At this time, the dog weighed 7.65 kg with a BCS of 5/9 and physical examination was unremarkable. A serum biochemistry profile, pre- and postprandial bile acid concentrations, ammonia tolerance testing, and a coagulation profile were within reference intervals. Urinalysis disclosed mild proteinuria (UPC, 0.51; RI, 0–0.3). On abdominal radiographs and ultrasound examination, the liver appeared small but otherwise unremarkable. Histopathologic evaluation of ultrasound-guided liver biopsy samples (16 G) identified mild fibrocytic proliferations and interlobular radiating reticulin fibers. The sinusoidal reticulin fiber network was no longer apparent (Fig 3). Small foci of hepatocytes displayed mild foamy cytoplasmic vacuo-
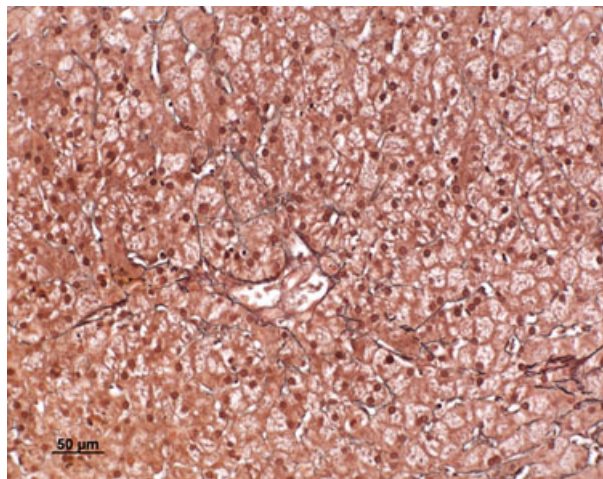
Fig 3. Photomicrograph of a silver-stained hepatic section of a Beagle (Case 1) with hereditary cobalamin malabsorption 1 year after diagnosis and treatment showing mildly increased interlobular reticulin fibers, but markedly reduced sinusoidal fiber deposition than before treatment.
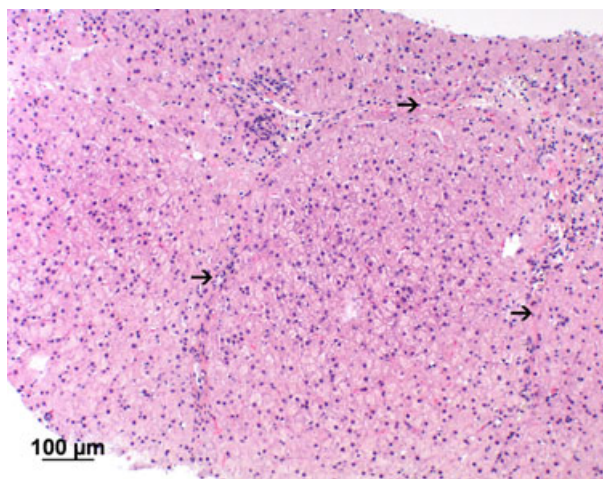

Fig 4. Photomicrograph of a hematoxylin-eosin–stained liver section of a Beagle (Case 1) with hereditary cobalamin malabsorption 1 year after diagnosis and treatment showing mild proliferations of fibrocytes and reticulin fibers radiating between some liver lobules (slender arrows).

lation (Fig 4). The interpretation was mild hepatic fibrosis. Serum cobalamin (350 ng/L; RI, 261–1,001 ng/L) and plasma homocysteine (6 μmol/L; RI, 4.3–18.4 μmol/L) levels were within reference intervals, and urine methylmalonic acid was undetectable. Weekly cobalamin supplementation (50 μg/kg IM) was continued. The owner reported the dog to be in good health at the time of writing (5.5 years after initial presentation).

Case 2

The medical records and biopsies of a full sibling of the dog described above were examined and are described here. A 6-month-old, male, intact Beagle was presented to a private referral clinic for progressive lethargy, anorexia, vomiting, and failure to gain weight. At presentation, the dog weighed 6.4 kg with a BCS of 4/9. Physical examination disclosed marked lethargy. A CBC identified marked neutropenia (770/μL; RI, 2,496–7,437/μL). A serum biochemistry profile, bile acid concentrations and cTLI were unremarkable. Urinalysis disclosed proteinuria (UPC, 0.59). No parasites were detected on fecal examination. Gastroduodenoscopy did not identify any gross abnormalities. Histopathologic evaluation of endoscopic gastric and duodenal biopsies was unremarkable with rare *Helicobacter* sp. organisms on the gastric mucosa.

After initial treatment with amoxicillin, metronidazole, and ranitidine, the dog's general condition and appetite improved. However, anorexia recurred on day 27 and prednisolone[d] (1.5 mg/kg PO q24h) was added to the treatment regimen. The dog's appetite improved, and by day 75, prednisolone was tapered over a week and discontinued. On day 128, the dog was presented again for anorexia and an enlarged abdomen. A CBC and biochemistry profile identified marked neutropenia (1,120 μL; RI, 2,496–7,437/μL), macrocytosis (MCV, 78 fL; RI, 64–73 fL), mild hypoproteinemia (4.6 g/dL; RI, 5.2–8.2 g/dL), mild hypoalbuminemia (2.0 g/dL; RI, 2.3–4.0 g/dL), moderate hypocholesterolemia (62 mg/dL; RI, 110–320 mg/dL), markedly increased ALP activity (857 IU/L; RI, 23–212 IU/L), and markedly decreased BUN concentration (2.0 mg/dL; RI, 7.0–27.0 mg/dL). Analysis of abdominal fluid was consistent with a pure transudate (total protein, <1.0 g/dL; nucleated cell count, 65 cells/μL). The serum cobalamin concentration was below the detection limit (<150 ng/L; RI, 261–1,001 ng/L). However, this result was interpreted as being indicative of bacterial overgrowth or small intestinal damage. An exploratory laparotomy identified ascites and a small liver with adequate portal vasculature. Approximately 1.5 L of peritoneal fluid was removed, and hepatic and duodenal biopsy samples were harvested.

Histopathologic examination identified mild edema and mildly increased numbers of eosinophils in the duodenal mucosa.[f] A panlobular distribution of small foci of swollen hepatocytes with either foamy cytoplasm and glossy nuclei or small clearly demarcated vacuoles was noted on hepatic sections (Fig 5). Fine lipofuscin deposition was observed in some hepatocytes and Kupffer cells. Occasional hepatocellular necrosis and mild lymphoplasmacellular and neutrophilic infiltration were observed. Immunohistochemistry[g] identified a few sinusoidal myofibroblast–like cells expressing smooth muscle actin (Fig 6). Gömöri staining[b] disclosed mild proliferation of sinusoidal reticulin fibers (Fig 7). The interpretation was moderate liver cell degeneration with single-cell necrosis, mild fibrosis, and secondary mild chronic lymphoplasmacytic and neutrophilic hepatitis. Despite continued supportive treatment, the dog's general condition deteriorated, only repeated drainage of reaccumulating ascites ameliorated clinical signs, and it was euthanized at the owner's request on day 150.
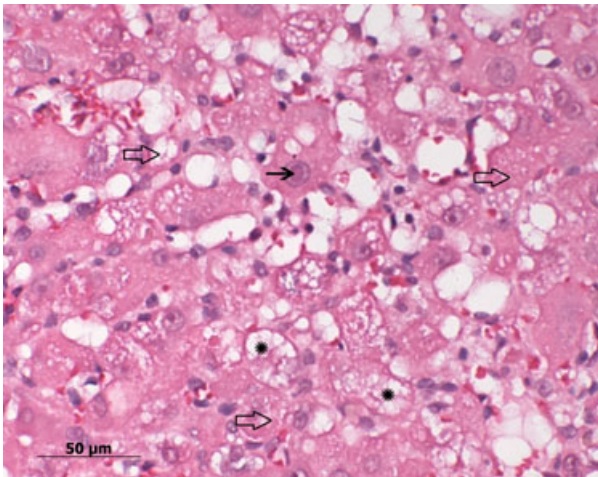
Fig 5. Photomicrograph of a hematoxylin-eosin–stained liver section in a Beagle (Case 2) with hereditary cobalamin malabsorption showing panlobular small foci of swollen hepatocytes with foamy cytoplasm (glycogen storage, asterisks). The nuclei of the hepatocytes are often round and swollen and show a glossy appearance (glycogen deposition caused by degeneration, slender arrows). Fine, slightly granular pigment (lipofuscin, broad open arrows) is visible in a few hepatocytes and Kupffer cells.
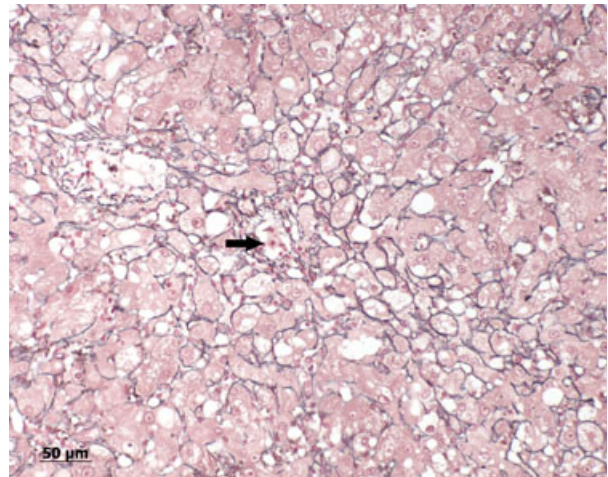

Fig 6. Photomicrograph of a liver section stained with anti–smooth-muscle actin (SMA) antibody in a Beagle (Case 2) with hereditary cobalamin malabsorption showing increased numbers of SMA-positive myofibroblast-like cells within the hepatic sinusoids.

Discussion

We describe 2 Beagle siblings with genetically confirmed hereditary cobalamin malabsorption that developed degenerative liver disease. Both dogs had a chronic history of lethargy, anorexia, and failure to gain weight. Neutropenia had been noted in both dogs and, mild macrocytosis in the absence of anemia was detected in 1 dog at some point. These are typical findings described in children with congenital cobalamin deficiency.[2,14] Neutropenia was also reported in the

Fig 7. Photomicrograph of a Gömöri-silver–stained liver section in a Beagle (Case 2) with hereditary cobalamin malabsorption showing increased numbers of reticulin fibers around the central veins, radiating into the hepatic sinusoids (broad arrow).
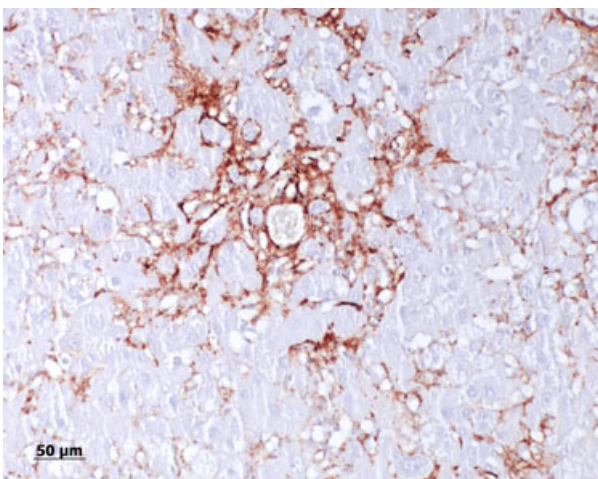
only previously published case of a cobalamin-deficient Beagle.[4] Because cubilin is required for renal tubular reabsorption of some proteins, persistent proteinuria is also a typical finding in children with IGS[15] and was detected in 1 dog in this report.

Hypocobalaminemia was recognized in Case 1, and the dog experienced a full clinical recovery despite mild residual hepatic lesions. In contrast, cobalamin supplementation was not given to the other dog, in which the disorder was progressive.

Degenerative hepatic disease has not thus far been described in association with cobalamin malabsorption in dogs, although laboratory evidence of hepatic dysfunction was evident in previously reported cases in Giant Schnauzers, a Beagle, and Border Collies.[4,6,8,11] The cause of hepatic dysfunction was not further investigated, presumably because of rapid clinical response to cobalamin supplementation. It is possible that the Beagles described in this report were affected by a hepatopathy independent of cobalamin deficiency. This, however, is unlikely based on sustained clinicopathologic and histologic improvement after cobalamin supplementation and withdrawal of other medications in Case 1 despite the negative prognostic factor of ascites,[16] and the fact that Beagles are not known to suffer from juvenile chronic liver disease. Hepatic disease in these dogs may have been caused by hyperhomocysteinemia, which develops because cobalamin is a coenzyme in the remethylation of homocysteine to methionine. Homocysteine has been suggested to cause hepatic damage in mice by oxidative stress, endoplasmatic reticulum stress, and activation of proinflammatory factors.[17,18] Furthermore, hyperhomocysteinemia promotes hepatic inflammation and fibrosis in rats.[19] Stellate cells produce collagen types I, III, and IV, which are deposited as a delicate reticulin network in the space of Disse during chronic hepatic injury.[20] These cells eventually transform into

smooth muscle actin–expressing myofibroblast-like cells,[20] which may explain the progression of liver pathology in Case 1. In both cases, minimal inflammation was apparent in biopsy specimens. This finding may also be linked to hyperhomocysteinemia secondary to cobalamin deficiency, because it has been shown experimentally that hyperhomocysteinemia induces expression and synthesis of monocyte chemoattractant protein-1 and other mediators of inflammation such as nuclear factor kappa B, interleukin (IL)-1b, IL-6, and IL-8 in liver tissue homogenates, suggesting that homocysteine may contribute to chronic inflammation in the liver.[17,21] Degenerative liver disease is well described in lambs and goats with decreased cobalt intake and hypocobalaminemia.[12,13,22] Features of ovine white liver disease are fatty changes, hepatocellular degeneration around central veins, and lipofuscin accumulation in hepatocytes and Kupffer cells.[12,22] Formation of lipofuscin suggests a role for lipid peroxidation, which has also been shown to be initiated by increased concentrations of homocysteine.[23]

Abdominal effusion with a pure transudate and normal or mildly decreased serum albumin concentration, observed in both dogs, suggests presinusoidal portal hypertension. This may be caused by changes in the extracellular matrix of the space of Disse and proliferation of myofibroblast-like cells expressing actin filaments as clearly seen in Case 2 (Fig 6). The changes may convert hepatic sinusoids from fenestrated channels to those of higher resistance with limited solute exchange.[20]

Treatment with corticosteroids in Case 1 was prescribed because of the mild inflammatory infiltrate in the liver biopsies. At this time, a possible link between hepatic pathology and cobalamin deficiency was not recognized. In retrospect, treatment with corticosteroids may have been unnecessary, but additional cases are needed to better characterize the prognosis and ideal treatment recommendations for this condition. In conclusion, we describe the clinical findings in Beagle siblings with genetically confirmed cobalamin malabsorption that developed extensive degenerative liver disease. Clinicopathologic findings included transient macrocytosis, neutropenia, and evidence of liver dysfunction. Clinical signs and hepatic lesions improved with cobalamin supplementation in the treated Beagle. Clinicians should consider hereditary cobalamin malabsorption as a differential diagnosis in young dogs with failure to thrive, and evidence of liver disease.

Footnotes

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[b] Modified Gömöri staining: Silver staining using routine protocol
[c] Vitamin B 12 Amino; Amino AG, Neuenhof, Switzerland
[d] Prednisolone 5 mg; Streuli Pharma AG, Uznach, Switzerland
[e] Denosyl 90 mg; Nutramax Laboratories Inc, Edgewood, MD
[f] Day MJ, Bilzer T, Mansell J, Wilcock B, Hall EJ, Jergens A, et al. Histopathological standards for the diagnosis of gastrointestinal inflammation in endoscopic biopsy samples from the dog and cat: a report from the World Small Animal Veterinary Association Gastrointestinal Standardization Group. World Small Animal Veterinary Association Gastrointestinal Standardization Group. J Comp Pathol 2008;138(Suppl 1):S1-43
[g] Immunohistochemistry: using a monoclonal mouse anti-smooth-muscle actin antibody (Dako N1584) at room temperature for 30 min without pretreatment. A peroxidase/AEC labeled secondary anti-mouse antibody (detection kit from Dako, K 5003) was used. Dako Schweiz AG; Baar, Switzerland

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