

## ***Lawsonia intracellularis* in a dog with inflammatory bowel disease**

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**ABSTRACT:** A two-year-old male German short-haired pointer was presented with a 1.5-year history of intermittent small-bowel diarrhoea. Inflammatory bowel disease (chronic lymphocytic-plasmacytic gastritis, enteritis and colitis) was diagnosed on the basis of histological examination of biopsies obtained on repeated endoscopy and by exclusion of other possible causes. Warthin-Starry silver staining of stomach mucosa revealed the presence of gastric spiral organisms. The evidence of *L. intracellularis* was provided by a positive nested polymerase chain reaction in one biopsy of duodenal mucosa and in one rectal smear. In 5 blood sera collected over a period of 8 months the IgG antibodies to *L. intracellularis* were found by an indirect fluorescent antibody test. Treatment with oral prednisone led only to a temporary improvement.

**Keywords:** *Lawsonia intracellularis*; dog; diarrhoea; chronic gastritis; enteritis; colitis; inflammatory bowel disease; endoscopy; nested polymerase chain reaction

*Lawsonia intracellularis* is now considered to be a well-established pathogen in pigs (Lawson and Gebhart, 2000). It is the cause of proliferative enteropathy, a condition characterised by a thickening of the mucous membrane of the intestine with proliferation and immaturity of the intestinal epithelium. The disease has a worldwide distribution and typically causes diarrhoea and growth retardation (Rowland and Lawson, 1992). In addition to pigs, proliferative enteritis associated with *L. intracellularis* has been described in numerous animal species (Cooper and Gebhart, 1998). Only two cases have been reported in the dog. Both reports preceded the description of *L. intracellularis* by McOrist *et al.* (1995) as a novel species of bacterium and the agents were referred to as *Campylobacter*-like organisms

(CLO). Collins and Libal (1983) described proliferative enteritis in two Dalmatian pups from one litter that had died from chronic watery diarrhoea and vomiting. In one of the pups, Warthin/Starry-stained sections of ileal mucosa revealed intracytoplasmic bacteria with ultrastructural features of CLO. Leblanc *et al.* (1993) have found CLO in a 7-month-old female Beagle dog with hyperplastic gastritis. While the presence of *L. intracellularis* in the former case was not unequivocally confirmed, the agent found in the latter dog was characterised well enough by a reaction with polyclonal rabbit antiserum (1080/76) against the omega antigen of *L. intracellularis*. This can therefore be considered the first convincing evidence of *L. intracellularis* infection in dogs (Lawson and Gebhart, 2000).

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The present report describes a clinical case of inflammatory bowel disease in a dog where *L. intracellularis* presence has been documented using nested polymerase chain reaction (PCR) and serology. Details on methodological aspects of these and some other laboratory examinations have been given elsewhere (Tomanová *et al.*, 2003).

## MATERIAL AND METHODS

### History, physical and ultrasound examination

A two-year-old male German short-haired pointer was presented with a 1.5-year history of intermittent diarrhoea of gradual onset. The dog passed large volumes of semiformed faeces of beige to greenish colour, and with foul odour. The frequency of defecation decreased from 5–6 times daily during the initial period of disease to 3–4 times daily at the time of presentation. The owner also noticed polyphagia, borborygmus and sometimes tenesmus. Neither raw pork meat nor organs were ever included in the dog's diet. The dog was temporarily placed on a commercial controlled diet (Eukanuba Lamb and Rice; IAMS and later Intestinal Formula; IAMS). The owner noticed only mild improvement when feeding the diet. The therapy with oral amoxicillin-clavulanate (Synulox 500 tbl. ad us. vet.; Pfizer) of one month's duration instituted by another veterinarian ten months before our initial examination also led only to slight improvement according to the owner. On

physical examination (day 0), the dog appeared bright, alert and in good bodily condition. The jejunal loops appeared slightly thickened on abdominal palpation. No other significant clinical abnormalities were detected. Ultrasound evaluation revealed decreased intestinal peristalsis, mild fluid retention in the duodenum and in a part of the jejunum with dilatation of intestinal lumen to approximately 5 mm. The duodenal wall thickness was 5 mm and that of jejunum 4 mm, both at the upper limit of normal range. Mesenteric lymph nodes were not enlarged.

### Clinical pathology and parasitology

Routine haematological evaluation revealed eosinophilia ( $1.729 \times 10^9$  eosinophils/litre; reference range 0 to  $0.75 \times 10^9$ /litre). The remaining parameters including thrombocyte counts were within the normal range. On repeated examination 7 months later, the eosinophilia decreased to  $0.872 \times 10^9$  eosinophils/litre. Serum biochemistry was normal except for an increased amylase and lipase activity ( $36.55 \mu\text{kat}$  per litre and  $12.06 \mu\text{kat}$ /litre, respectively, reference values being under  $17 \mu\text{kat}$ /litre for amylase and under  $8.4 \mu\text{kat}$ /litre for lipase). The trypsin-like immunoreactivity (TLI) examination, performed by Drs D.A. Williams and J.M. Steiner at the GI Lab, Department of Small Animal Medicine and Surgery, Texas A&M University, U.S.A, was normal on both initial and repeated examination 6 months later ( $8.4 \mu\text{g}$ /litre and  $12.7 \mu\text{g}$ /litre, respectively). Parasitological examinations of faeces repeated

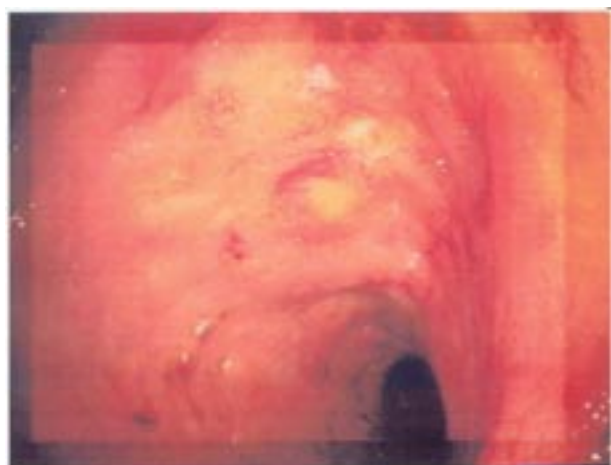


Figure 1. View of the duodenum with hyperaemic mucosa, pale foci of suspect mucosal infiltration, mild diffuse bleeding and increased granularity

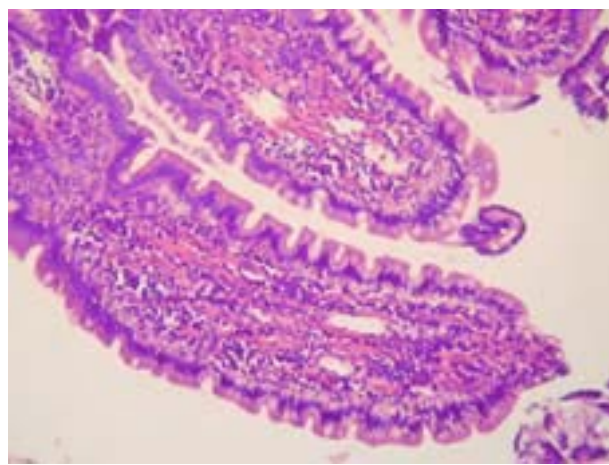


Figure 2. Photomicrograph of a section of duodenum demonstrating the infiltration of *lamina propria* with lymphocytes (H and E – 400 $\times$ )

three times every other day were negative initially and 8 months later.

### Microbiology

Bacterial culture aimed at *Campylobacter* spp., *Salmonella* spp. and *Clostridium* spp. was negative as well. Cytological examination of a rectal smear revealed only few *Clostridium* spores. Bacteriological tests for *Helicobacter* spp. yielded negative results.

### Endoscopy

Gastroduodenoscopy was performed on day 40 and 217 and colonoscopy on day 237 from the initial examination. Multiple endoscopically guided pinch biopsies were collected at the stomach, duodenum, colon and ileum (6, 6, 6 and 4 samples, respectively). Endoscopy performed on day 40 and 217 revealed foci of suspect mucosal infiltration with small non-bleeding erosions in the pyloric antrum and red stripes radiating from the pyloric orifice, probably caused by the manipulation with the endoscope. The duodenal mucosa was hyperaemic with pale foci, slightly increased granularity, friability and bleeding tendency during biopsy (Figure 1). Numerous erosions and foci of suspect mucosal infiltration were observed also on colonoscopy (day 237), particularly in the colon descendens. The ileum could not be evaluated visually, although blind biopsies were obtained.

### Histopathology

Histological examinations of the biopsies revealed discrete inflammatory infiltration of gastric mucosa with lymphocytes and plasmacytes, mild chronic duodenitis with lymphocytic-plasmacytic (Figure 2), and sporadically also polymorphonuclear infiltration. Sporadic local erosions and signs of the activity of an inflammatory process were noted in the duodenum. Moderately severe chronic lymphocytic-plasmacytic colitis with foci of inflammatory activity (focal presence of polymorphonuclear leucocytes) and exceptional small erosions were observed in the colonic biopsies. Lymphocytic infiltrate and erosions were observed also in biopsies from the ileum. At the sites of the presence of gastric bacteria, foci of lymphocytes and

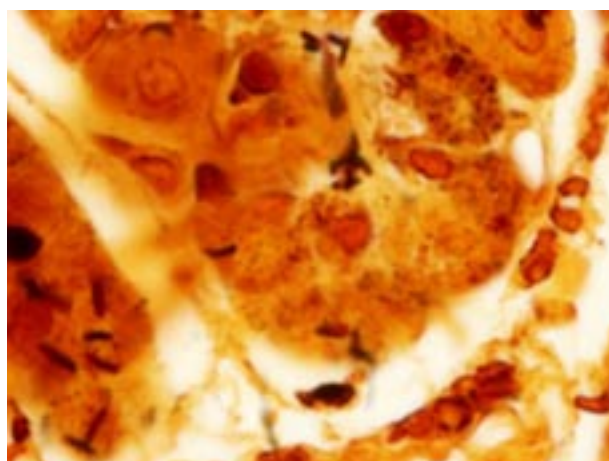


Figure 3. Silver-stained gastric spiral organisms in a section from the mucosa of the stomach (Warthin-Starry – 1 000×)

shallow mucosal erosions were observed. Periodic acid-Schiff (PAS)-staining was negative. The diagnosis can be summarised as inflammatory bowel disease (IBD), namely lymphocytic-plasmacytic gastro-entero-colitis. In one of the eight biopsies subject to extended examination (collected on day 217), Warthin-Starry silver staining of sections from the mucosa of the stomach revealed gastric spiral organisms (length 7–9  $\mu\text{m}$ ) with prevailing extracellular, partly also inter- and intracellular location (Figure 3). The bacteria were negative (non acid-fast) in modified Ziehl-Neelsen staining.

### Polymerase chain reaction and serology

The evidence of *L. intracellularis* was provided by the nested polymerase chain reaction (PCR) technique. Of the 8 gastrointestinal biopsies examined, one sample from the duodenum was positive (collected on day 217). Fourteen rectal smears were also examined by the nested PCR technique over a period of 8 months, with one sample being positive (collected on day 0). In all 5 samples of blood sera collected over a period of approximately 8 months and examined by serum indirect fluorescent antibody test (IFAT), the presence of IgG antibodies was found at the titre of 30 and 100.

### Therapy

From day 41 the dog was treated with oral prednisone (Prednison 20 Léčiva tab.) at a dose of 1 mg

per kg b.w. bid administered for 2 weeks, followed by 1 mg/kg sid and then tapering that dose for a total of 6 weeks. Following the marked initial improvement, the diarrhoea relapsed after the medication was withdrawn.

## DISCUSSION

To our knowledge, the presence of *L. intracellularis* in dogs has never been confirmed by DNA techniques. In the present case, only two of many samples (biopsies and rectal smears) collected over a relatively long period were positive. The latter was probably due to irregular shedding of low numbers of *L. intracellularis* in faeces. On the other hand, all blood samples collected over a relatively long period were serologically positive.

The absence of typical proliferative changes and intracellular bacteria with characteristic morphological features of *L. intracellularis* in our patient differ from the findings described in dogs by Collins *et al.* (1983) and Leblanc *et al.* (1993). Lesions caused by *L. intracellularis* in other animal species differ in location and histological detail, but all show the epithelium proliferation and presence of intracellular bacteria. Inflammatory changes may be involved at a later stage of the process. While inflammatory cell accumulations are not typical of the disease in pigs (Rowland and Lawson, 1992), the intestinal lesions in horses were classified as multifocal, lymphocytic, proliferative enteritis. The inflammatory infiltrate was composed of large numbers of lymphocytes and plasma cells (Brees *et al.*, 1999). Also in acute fatal outbreaks in monkeys, inflammatory changes were a marked feature (Klein *et al.*, 1999). In most species, the ileum and sometimes jejunum or proximal colon are the most frequently affected parts of the intestine by proliferative enteritis (Rowland and Lawson, 1992; Lavoie *et al.*, 2000). In the case reported here we were unable to visualise the ileum during coloscopy and canine jejunum is generally inaccessible endoscopically. We thus cannot exclude the presence of proliferative lesions or intracellular bacteria in these parts of the intestines.

The idiopathic inflammatory bowel diseases (IBD) are a group of disorders characterised by persistent clinical signs of gastrointestinal disease associated with histological evidence of inflammation of undetermined cause in the lamina propria of the intestine. By definition, diagnosing IBD means ruling out the known causes of gastrointestinal

inflammation, of which there are many (Guilford, 1996). These criteria were fulfilled in this case. The gastrointestinal tract was affected by chronic lymphocytic-plasmacytic inflammation at multiple sites (stomach, duodenum, ileum, colon) and perhaps along its entire length. Lymphocytic-plasmacytic enteritis and colitis represent the most common forms of IBD in dogs (Guilford, 1996). Although the dog improved slightly and temporarily on a controlled diet, this was not sufficient for the diagnosis of dietary sensitivity. The repeated TLI assay excluded exocrine pancreatic insufficiency. The mild increase of amylase and lipase activity was nonspecific and was likely caused by the gastrointestinal inflammation. Parasites and common important enteric pathogens were excluded by repeated examinations. Thus the finding of *L. intracellularis* raised the possibility that this bacterium might be the cause of IBD in our patient. The responsiveness of the inflammatory bowel disease to immunosuppressive therapy strongly rules against an infectious cause in most cases of human and animal IBD (Strober and James, 1986). Nevertheless, in a foal with *L. intracellularis* proliferative enteropathy (Frank *et al.*, 1998), the treatment with corticosteroids provided a benefit as diarrhoea and protein loss resolved 2 days after the initiation of dexamethasone therapy. Thus the effect of corticosteroid treatment in this dog cannot rule out the infectious cause.

It is not possible to answer with certainty whether the presence of *L. intracellularis* caused the disease in our patient or was simply a concomitant or secondary infection. Since no concerted effort has ever been made to search for *L. intracellularis* in canine IBD, this finding indicates the need for further investigation of *L. intracellularis* in dogs.

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