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CANINE CHRONIC ENTEROPATHIES : EPIDEMIOLOGICAL STUDY, AND NEW PROGNOSTIC AND THERAPEUTIC ASPECTS

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DEDICATION

To my family and friends,
to be always on my side and for making me become the person I am.

To my teachers,
for teaching me to love my work and to desire the best for my career.

To my life,
full of adventures ready to be lived!

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NOMENCLATURE

CHRONIC ENTEROPATHIES NOMENCLATURE

CE	Chronic enteropathy, include FRE, ARE, IBD
FRE	Food responsive enteropathy
ARE	Antibiotic responsive enteropathy
IRE	Immunosuppressant-responsive enteropathy
SRE	Steroid responsive enteropathy, term used instead of IRE
NRE	Non responsive enteropathy
IBD	(Idiopathic) Inflammatory bowel disease, include IRE, SRE and NRE
PLE	Protein loosing enteropathy

OTHER ABBREVIATIONS:

(q)PCR	(quantitative) Polymerase chain reaction
ACTH	Adrenocorticotropic hormone
AHD	Acute hemorrhagic diarrhea
ALT	Alanine aminotransferase
ARF	Adverse reaction to food
AST	Aspartate aminotransferase
AT III	Antithrombin III
BA	Bile acids
BARF	Bone and Raw Food diets
BCS	Body condition score
BID	*Bis in die*, twice daily, q12h
BW	Body weight
CCECAI	Canine CE clinical activity index
CIBDAI	Canine IBD activity index
CIPO	Chronic intestinal pseudo-obstruction
cPLI	Canine pancreatic lipase immunoreactivity
CRP	C reactive protein
EE	Eosinophilic enteritis
EPI	Exocrine pancreas insufficiency
FC	Fecal score
FISH	Fluorescent in situ hybridization
FOS	Fructooligosaccharides
GI	Gastrointestinal
GSD	German shepherd dog
GSE	Gluten sensitivity enteropathy
HC	Healthy control;
IBS	Irritable bowel syndrome
ICRP	Inflammatory colorectal polyps
IV	Intravenously
LPE	Lymphocytic plasmatic enteritis
LS	Lundehund syndrome
MCS	Muscle condition score
MOS	Mannanoligosaccharides
MSC	Mesenchymal stem cells
NGS	Next generation sequencing
NHD	Non-hemorrhagic diarrhea
NLR	Nod like receptor
PCV	Packet cell volume
PLN	Protein loosing nephropathy
PO	*Per os*, oral route
PSS	Porto-systemic shunt.
PU/PD	Polyuria polydipsia
RBC	Red blood cell
SAP	Serum alkaline phosphatase
SBS	Short bowel syndrome
SC	Subcutaneous
SCWT	Soft Coated Wheaten Terriers
SIBO	Small intestinal bacteria overgrowth
SID	*Solum in die*, once a day, q24h
T-RFLP	Terminal restriction fragment polymorphism
TEG	Thromboelastography
TIBC	Total iron binding capacity
TID	*Ter in die*, three times daily, q8h
TLI	Trypsin-like immunoreactivity
TLR	Tool like receptor
UPC	Urinary protein to creatinine ratio
US	Ultrasound or ultrasonography
USG	Urine specific gravity
WBC	White blood cell
WHWT	West Highland White Terrier
α1-PI	α1-Protease inhibitor

INTRODUCTION

Chronic enteropathy (CE) is a term used for diseases of the intestines regardless of etiology and pathogenesis, and it is otherwise called *inflammatory bowel disease* (IBD). In humans IBD, includes two different chronic disorders characterized by inflammation of the intestinal wall: Crohn's disease (CD) and ulcerative colitis (UC). Like humans, dogs frequently develop CE and it is considered the main cause of chronic, persistence or episodic gastrointestinal signs (GI).[1] Similar to human IBD, the combination of underlying host genetic susceptibility, inappropriate immune responses and uncontrolled inflammation, altered intestinal microbiota (dysbiosis), and dietary and/or environmental factors are suspected as main contributing factors in the pathogenesis of canine IBD.[1,2]

CE in dogs can further be subdivided retrospectively by response to treatment into: *food-responsive enteropathy* (FRE), *antibiotic-responsive enteropathy* (ARE), *immunosuppressant-responsive enteropathy* (IRE) otherwise called *steroid-responsive enteropathy* (SRE) or *idiopathic* IBD, and *non-responsive enteropathy* (NRE).[1,3] In addition to this classification, according to treatment response, dogs with loss of protein across the gut are typically grouped as *protein-losing enteropathy* (PLE), highlighting the more guarded prognosis of this particular form of CE compared to dogs with normal serum albumin concentration.[1,3,4] PLE can be a consequence of inflammation or neoplastic disease. PLE dogs can potentially be FRE or ARE, but usually are IRE.

The purpose of this thesis is to review the literature in matter of chronic enteropathies. Moreover, studies conducted during the author's PhD were reported. Investigations were focused on the different aspects of the disease, from etiopathogenesis (chapter 1.1), breed or sex predisposition in Italian population (chapter 2.1), clinical signs associated with the disease (chapter 3.1), treatment options (chapter 5.1, 5.2, 5.3, 5.4), and, finally, identification of prognostic factors (chapter 6.1, 6.2).

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1. PATHOGENESIS

Pathogenesis of CE is generally believed to be multifactorial. A currently accepted hypothesis in humans, dogs, and cats is that a dysregulation of the gastrointestinal immune system in genetically susceptible individuals, may lead to aberrant responses towards luminal microbial or dietary antigens.[1–3]

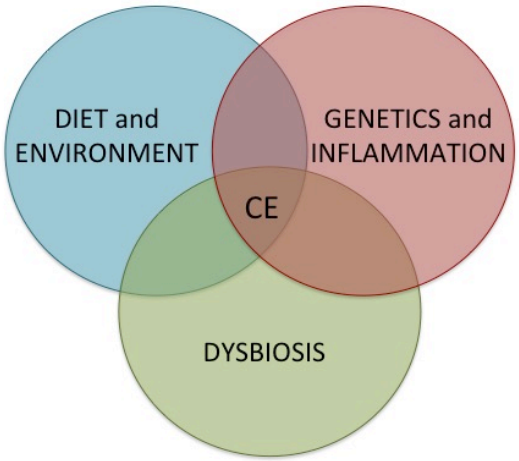

Figure 1.a: Factors involved in the pathogenesis of CE.

INFLAMMATION AND GENETIC PREDISPOSITION

Inflammation plays a crucial role in the pathogenesis of CE.[2,4] Tolerance of dietary and GI microbial antigens (*oral tolerance*) is achieved following interactions between the intestinal barrier and epithelial tight junctions, phagocytes, and inflammatory cells.[3,5] Loss of oral tolerance leads to uncontrolled inflammation, which is the result of activation of the many effector pathways. The inflammation can then lead to architectural disruption, resulting in adverse effects on function.[3,5]

Toll-like receptors (TLRs) and NOD-like receptors (NLRs) are ideally situated on intestinal epithelia cells and recognize microbe associated molecular patterns (MAMPs).[6] TLRs may play a significant role in defense against pathogens, but inappropriate activation of their signaling pathways may lead to deleterious inflammation and tissue injury.[7] In genetically predisposed individuals, TLRs and NLRs have been shown to be up-regulated in the intestines of human beings with Crohn disease, ulcerative colitis, celiac disease, and colon cancer.[7] Similarly, TRLs 2, 4, and 9 were found to be up-regulated in dogs with chronic enteropathies.[6,8,9] A genetic predisposition for genes encoding TRL 4 and 5 was demonstrated in German Shepherd dog (GSD) and also in other breeds, underlying the importance of genetics and immune-mediated mechanisms in the pathogens of CE.[2,8,10]

Although little information is currently available in companion animals, several studies have characterized the cells and cytokines expressed within the canine and feline GI tract in both healthy animals and those with CE.[3] Studies reveal different expression of cytokines, different *lamina propria* T and B cell distribution, increased densities of different IgA, IgG plasma cells, T cells, MHC class II+ cells, and macrophages [e.g in GSD with IBD and Boxer with histiocytic ulcerative colitis (HUC)]. [2,3,11–13]

Overall, there is a lack of an obvious pattern of cytokines expression, cell distribution, genetic predisposition, or expression of single TLR (or combination of TLRs) in dogs with CE. Therefore, in summary, recent studies provide evidence that both genetics and inflammation have a role in the development of CE in dogs and cats, but exact mechanisms remain elusive and further studies are needed.

DYSBIOSIS

DEFINITIONS OF -OMES

The term *microbiome* initially was used to describe the "the ecological community of commensal, symbiotic and pathogenic microorganisms that literally share our body space"; nowadays it refers to the entire genetic mass (*genome*) of microorganisms, including *bacteriome*, *virome* and *mycobiome* (Figure 1.b).[14–17]

The term *microbiota* (in the past also referred to as *microflora*) is used to describe bacterial communities on mucosal surfaces (with or without luminal microorganisms) or on other body sites (e.g. skin). The intestinal microbiota is, therefore, the collection of microorganisms (bacteria, fungi, protozoa, and viruses) inhabiting the gastrointestinal tract (Figure 1.b).[18,19]

Metagenomics is the study of the *metagenome* (the collective genome of microorganisms from an environmental sample) and provide information on the microbial diversity and ecology of a specific environment (Figure 1.a).[20]

Metabolomics refers to the systematic identification and quantification of the metabolic products (the *metabolome*) of a biological system (cell, tissue, organ, biological fluid, or organism) at a specific point in time.[21] Metabolomics does not necessarily refer to microbiota metabolites, but also to other metabolites (es. bile acids, short chain fatty acids). Study of microbiota and metabolomics can therefore aid in comprehension of the pathophysiology of GI system in health and disease and are subject of many studies..[21]

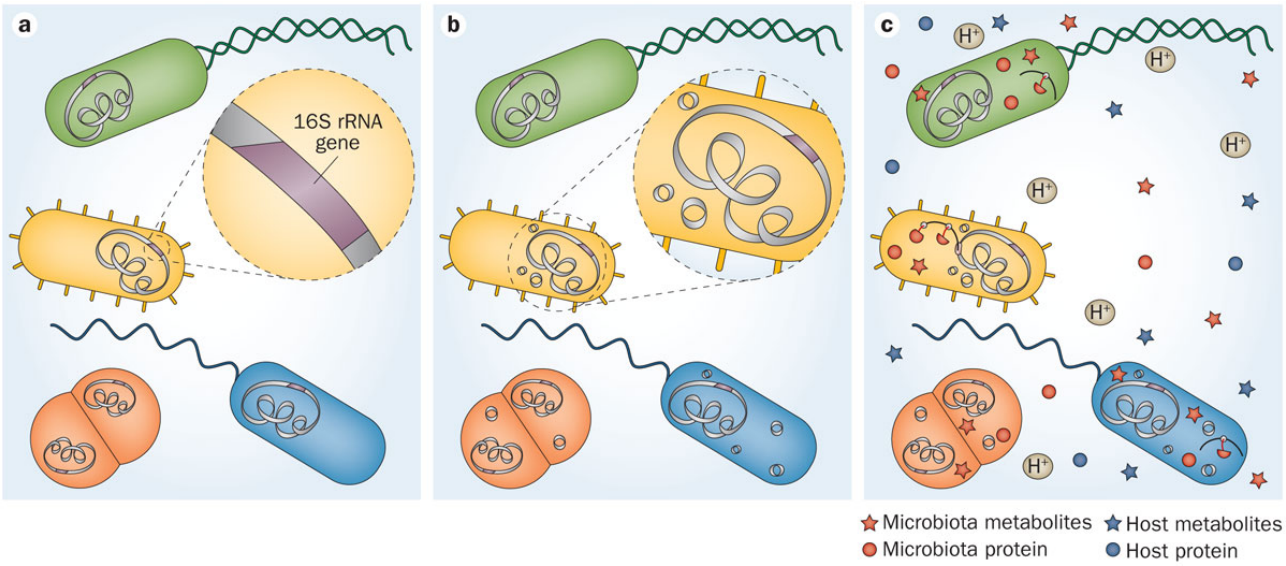

Figure 1.b: Definition of the microbiota, metagenome and microbiome. Each image represents the same population; however, different approaches to define the population provide different information. **a** *Microbiota*: 16S rRNA surveys are used to taxonomically identify the microorganisms in the environment. **b** *Metagenome*: the genes and genomes of the microbiota, including plasmids, highlighting the genetic potential of the population. **c** *Microbiome*: the genes and genomes of the microbiota, as well as the products of the microbiota and the host environment. From Whiteside et al, 2015.[22]

rRNA, ribosomal RNA.

INTESTINAL DYSBIOSIS

In recent years, the GI microbiota has garnered strong interest due to the potential ethiopathologic role in host health and disease. Intestinal dysbiosis can be defined as an alteration in the composition and/or richness of the intestinal microbiota. Studies in veterinary species have associated intestinal dysbiosis with various GI disorders, such as acute diarrhea, CE, granulomatous colitis, and colorectal polyps.[18,23–26] Whether these changes are a cause or a consequence of the aberrant immune reactions seen in the GI tract remains a matter of debate, both in people and in dogs.[15] There is likely an overlap, since inflammation will cause dysbiosis, and recent

functional studies have demonstrated that dysbiosis, when present, is a risk factor that may exacerbate inflammation in genetically susceptible individuals.[19]

There is no single gold standard for assessing the GI microbiota and dysbiosis. Most current research is focused on evaluating the bacterial microbiota and methods have been optimized for characterization of bacteria.[19] Bacterial culture can be a useful in specific situation (e.g. specific infection, antibiotic sensitivity testing), but in most cases it not useful. Since the gut microbiota is a complex and dynamic ecosystem, the best diagnostic approach would be a combination of molecular tools that include PCR amplification of 16S rRNA genes using broad universal bacterial primers, followed by analysis of amplicons by next generation sequencing (NGS), direct quantification of specific bacterial taxa by quantitative PCR (qPCR), and the use of fluorescent in situ hybridization (FISH) to visualize the translocation of bacteria into the mucosal epithelium.[19]

Many studies evaluate the alternations of microbiota in different samples and several techniques were evaluated for better understanding the composition of microbiota during acute and chronic GI disease in dogs. Results are reported in Table 1.a

References	Sampling location	Sample size	Method	Microbial changes
Suchodolski et al, 2012[23]	Duodenal biopsies	IBD (n = 14) HC (n=6)	454-pyrosequencing (16S rRNA gene)	Increase in Proteobacteria (*Diaphorobacter, Acinetobacter*) Reduction in *Fusobacteria*, *Bacteroidaceae*, *Prevotellaceae*, *Clostridiale*
Suchodolski et al, 2010[27]	Duodenal biopsies	IBD (n=7) HC (n=7)	Gene clone libraries (16S rRNA gene)	Increase in *Proteobacteria* Decrease in *Clostridia*
Allenspach et al. 2010[28]	Duodenal brushing	CE (n=13) HC (n=8)	Gene clone libraries (16S rRNA gene)	Increase in *Actinobacteria*, *Lactobacillales*, *Erysipelotrichales*
Xenoulis et al, 2008[29]	Duodenal brushing	IBD (*n* = 10) HC (*n* = 9)	Gene clone libraries (16S rRNA gene)	Increase in *Enterobacteriaceae* (*E. coli*); Reduction in biodiversity
Suchodolski et al, 2008[30]	Duodenal brushing	CE (*n* = 71) HC (*n* = 64)	Gene clone libraries (fungal ITS gene)	No significant differences in fungal communities
Glanemann et al, 2008[31]	Stomach, duodenum, Colon biopsies	Chronic GI disease (*n* = 42) HC (*n* = 14)	PCR	Presence of *Mycobacterium avium* subspecies *paratuberculosis* Detected in 8/42 (19%) of dogs with chronic GI disease
Manchester et al, 2013[32]	Colon biopsies	HUC (*n* = 6)	FISH	Presence of invasive *E. coli*
Simpson et al, 2006[33]	Colon biopsies	HUC (*n* = 6) HC (*n* = 38)	FISH	Intracellular translocation of adherent and invasive *E. coli*
Rossi et al, 2014[34]	Fecal samples	IBD (*n* = 20) HC (*n* = 10)	qPCR (16S rRNA gene)	Decreased in *Faecalibacterium* spp. And *Turicibacter* spp.
Foster et al, 2013[14]	Fecal samples	Acute diarrhea (*n* = 7) HC (*n* = 12)	454-pyrosequencing (18S rRNA gene)	No significant differences in fungal communities
Suchodolski et al, 2012[24]	Fecal samples	IBD (*n* = 19) AHD (*n* = 13) NHD (*n* = 12) HC (*n* = 32)	454-pyrosequencing (16S rRNA gene) qPCR (16S rRNA gene)	AHD: most profound alterations in their microbiome Increase in *Sutterella*, *Clostridium perfringens* Decrease in *Blautia*, *Ruminococcaceae*, *Turicibacter* IBD: Decrease in *Faecalibacterium* spp., *Fusobacteria*

References	Sampling location	Sample size	Method	Microbial changes
Jia et al, 2010[35]	Fecal samples	Chronic diarrhea (*n* = 9) HC (*n* = 8)	FISH	Increase in *Bacteroides*
Glanemann et al, 2008[31]	Fecal samples	Diarrhea (*n* = 4) HC (*n* = 9)	T-RFLP	Increase in *C. perfringens*, *E. faecalis*, and *E. faecium*

Table 1.a: Reported microbial shifts in dogs with gastrointestinal disease. Modified from: Honneffer et al, 2014.[18]
n, number of dogs; IBD: Inflammatory bowel disease; HC: Healthy control; HUC, histiocytic ulcerative colitis; AHD: Acute hemorrhagic diarrhea; NHD: Non-hemorrhagic diarrhea; CE: chronic enteropathies; FISH: Fluorescence in situ hybridization; T-RFLP: Terminal restriction fragment polymorphism; qPCR: Quantitative polymerase chain reaction.

FOOD INTOLERANCE AND ENVIRONMENTAL FACTORS

In humans, environmental risk factors for IBD are well characterized. Epidemiological and clinical evidence supports an association between IBD and many environmental factors such as: diet, oral contraceptive uses, smoking, geographical and social status, occupation, appendectomy, perinatal and childhood factors (hygiene, diet and mode of feeding, infections), vaccinations, and microbial factors.[36–38] Moreover racial differences have been documented, although studies of migrant populations suggest that ethnic and racial differences may be more related to lifestyle and environmental influences than true genetic differences.[38] In dogs, environmental factors are way less identified and are dietary habits, infectious diseases, stress and physical activity are thought to have a role.

In CE, adverse reactions to food (ARF) have a role in pathogenesis in all the phenotype of the disease. ARF is a common cause of gastrointestinal signs and can be divided into 2 major groups: immunologic (e.g., dietary hypersensitivity where an aberrant immune responses is involved) and non-immunologic (including food intolerance and dietary indiscretion).[39,40] Loss of oral tolerance is believed to be the cause of food intolerance or allergy. The initiating events that lead to loss of oral tolerance, or prevent it from developing, have not been described in dogs or cats, and remain poorly understood in any species.[40,41] Causes could be loss of mucosal integrity (e.g. injury or inflammation), parasitism, or dysbiosis. Loss of tolerance to dietary antigens will produce an aberrant immune response against the dietary antigen, lead to inflammation locally, or in another anatomical site (e.g cutaneaous manifestation or *otitis externa*).

Infections such as parvovirus infection, giardiasis, or other parasites are thought to be predisposing factors for development of CE. The hypothesis, as mentioned above, is that all these factors cause a damage to the intestinal wall, causing a breaches in oral tolerance. Besides these theories, data on this aspects is lacking. Finally, both physical activity and stress influence gut function. For example, acute diarrhea and gastric ulcers occurs frequently in sled-dogs[42] and in dogs in shelters,[43] but whether those factors predispose the dogs to CE is not known.

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1.1 RELATIONSHIP BETWEEN DUODENAL ENTEROCHROMAFFIN CELLS DISTRIBUTION AND IBD SEVERITY IN THE DOG

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ABSTRACT

Despite numerous studies carried out over the last 15 years in veterinary medicine, the pathogenesis of canine Inflammatory Bowel Disease is still not completely elucidated. In particular, unlike what has been demonstrated in human medicine, the influence of serotonin on clinical signs in canine Inflammatory Bowel Disease is not yet clarified. The objective of this paper has been to seek a possible correlation between duodenal epithelial distribution of serotonin-producing cells (enterochromaffin cells) and disease-grading parameters (clinical, clinico-pathological, endoscopic and histopathological) in dogs with Inflammatory Bowel Disease,

The medical records of dogs with a diagnosis of Inflammatory Bowel Disease were retrospectively reviewed and 21 client-owned dogs with a diagnosis of Inflammatory Bowel Disease were registered. Clinical score (by Canine Chronic Enteropathy Clinical Activity Index), laboratory exams (albumin, total cholesterol, folate, cobalamin), endoscopic score and histopathological score, were compared by regression with duodenal enterochromaffin cell percentage.

The study results suggest a relationship between a decrease in folate absorption and an increase in duodenal enterochromaffin cell percentage (regression equation $y=16.89-6.14x$; coefficient of determination r^2: 0.7; significant level: $P=0.007$). Meanwhile no significant relationship was evidenced between duodenal enterochromaffin cell percentage and the other analyzed variables.

***Key words**: dog, IBD, serotonin, enterochromaffin cells.*

INTRODUCTION

Canine Inflammatory Bowel Disease (IBD) is a disease based on evidence of clinical signs of chronic diarrhea and/or vomiting associated with weight loss, once excluded enteric infectious, parasitic, endocrine or neoplastic diseases, food responsive enteropathy (FRE) and antibiotic responsive diarrhea (ARD) (1).

For more than twenty years, IBD has been the major topic of discussion and research in canine gastroenterology and the interest in deepening its pathogenesis in the veterinary field is also related to the increasing knowledge that, currently, characterizes similar human diseases, such as Crohn's disease and ulcerative colitis.

Untill now, several studies about its ethio-pathogenesis and treatment have been performed in veterinary medicine, but little attention has been paid to the dysfunction of the regulation of the enteric nervous system that characterizes this syndrome.

Nervous control of gastrointestinal motility and secretion is a complex process, in which serotonin (5-HT) plays an important role (2).

The majority of 5-HT is stored in enterochromaffin (EC) cells included in gut epithelium, initiates peristaltic, secretory, vasodilatory, and nociceptive reflexes, and it is removed by serotonin-selective reuptake transporter (SERT), located on enterocytes, central or peripheral serotonergic neurons, and platelets (3, 4, 5). Altered serotonergic metabolism has been described in human gastrointestinal diseases (6, 7). In veterinary medicine, a recent report evidences a higher concentration of 5-HT and of the EC cell marker chromogranin-A (CgA) in the intestinal mucosa of dogs with IBD when compared with healthy controls (8).

Accordingly, we have evaluated, in dogs with IBD, the relationship between duodenal EC cell distribution and the degree of the clinical condition, the result of laboratory tests, endoscopic appearance and duodenal histology.

MATERIAL AND METHODS

The medical records of dogs with a diagnosis of IBD between January 2011 and November 2013 were retrospectively reviewed.

Twenty-one client-owned dogs were included (seven crossbred and 14 purebred – Rottweiler [n=2], Basset Hound [1], Bolognese [1], Boxer [1], Cocker Spaniel [1], Dachshund [1], Epagneul Breton [1], German Shepherd [1], Great Dane [1], Labrador Retriever [1], Maltese [1], Pointer [1], West Highlander White terrier [1]), 4 females (2 spayed) and 17 males (4 castrated), mean age of 5.8±3.2 years,.

The inclusion criteria embraced history, a physical examination, a complete blood count, a serum biochemistry profile, serum folate concentration, serum cobalamin concentration and a negative fecal flotation test or treatment with fenbendazole (Panacur, Intervet Italia Srl, Milano, Italy) 50 mg/kg SID for three days.

Persistence of clinical signs that followed a dietary modification (hypoallergenic diet) for three weeks was applied to exclude FRE, and treatment to exclude ARD was applied for 3 weeks with tylosin at 15 mg/kg, PO, q 12 h (Tylan, Eli Lilly Italia, Firenze, Italy) or metronidazole (Flagyl, Zambon Italia, Vicenza, Italy) at 10 mg/kg PO, q 12 h.

An abdominal ultrasound examination, a complete endoscopic examination of the alimentary tract, and a histological examination of enteric bioptic samples concluded the diagnostic process, arising from the diagnosis of IBD.

CLINICAL SCORE

In order to be later used for comparison, clinical data, obtained before treatment, were scored by the validated clinical score index (Canine Chronic Enteropathy Clinical Activity Index, CCECAI) (9).The analyzed parameters were attitude/activity, appetite, vomiting, stool consistency, stool frequency, body weight loss, serum albumin concentration, presence of peripheral edema/ascites and severity of pruritus.

The parameters were analyzed with a score moving from 0 (normal condition) to 3 (worst condition).

The total score from 0 to 3 points indicates an insignificant disease, from 4 to 5 points indicates a mild disease, from 6 to 8 points indicates a moderate disease, from 9 to 11 point indicates a severe disease, and a score ≥ 12 points indicates a very severe disease.

LABORATORY ANALYSIS

On serum samples collected before treatment, albumin (reference range [RR] 2.8-3.7 g/dL), total cholesterol (RR 140-350 mg/dL), serum folate (RR 6.5-11.5 µg/L) and serum cobalamin (RR 250-730 ng/L) were employed for further statistical analysis.

ENDOSCOPIC SCORE

Endoscopic video recordings of the duodenum, performed in digital manner during the diagnostic procedure, were revaluated and scored following the endoscopic activity score index (EASI) as reported by Slovak et al. (2015) (10).

In particular the endoscopic parameters, evaluated with a score from 0 to 2, were: friability (bleeding on contact with endoscope or biopsy forceps), granularity (alteration in the texture of the mucosa), erosions (superficial linear mucosal defect[s] with hemorrhage) and lymphatic dilatation (multifocal to diffuse white foci within the mucosa).

HISTOPATHOLOGICAL SCORE

Three to five duodenal biopsies, collected from each dog during the endoscopic examination, were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4µm, and stained with hematoxylin and eosin. Sections were re-examined in all dogs following the histopathological standard of the World Small Animal Veterinary Association (WSAVA) Gastrointestinal Standardization Group (11).

In particular the morphological criteria (villous stunting, villous epithelial injury, crypt distension, lacteal dilation, mucosal fibrosis, goblet cells) and the parameters of inflammation (intraepithelial lymphocytes, lamina propria lymphocytes and plasma cells, lamina propria neutrophils) were scored with a 0-3 scale (0, normal state; 1, mild; 2, moderate; 3, severe).

The final score was the sum of all the considered parameters.

IMMUNOHISTOCHEMICAL ANALYSIS

Immunohistochemistry was performed with a streptavidin-biotin-peroxidase technique (Biospa, Milano, Italy) in order to evidence the percentage of EC cells with respect to all the cells of duodenal epithelial layer.

Replicate 4µm-thick sections were cut from the paraffin block of each case, incubated with hydrogen peroxide 0.3% in methanol for 20 min to block endogenous peroxidase activity and microwaved in citrate buffer (pH 6.0), for two cycles of 5 min., for antigen retrieval. Sections were then incubated overnight at 4°C in a humid chamber with the primary antibody (polyclonal rabbit anti-human chromogranin-A, Dako, Glostrup, Denmark) diluted 1:500 in PBS (pH 7.4, 0.01 M). Following washing in PBS, sections were then incubated with secondary biotinylated anti-rabbit IgG for 30 min at room temperature, and subsequently with streptavidin-peroxidase complex for 25 min at room temperature. After incubation in DAB chromogenic substrate solution (diaminobenzidine 0.02%, and H_2O_2 0.001% in PBS) for 12 min, sections were immediately rinsed in PBS and in running tap water, counterstained with hematoxylin, dehydrated and mounted with DPX (Fluka, Riedel-de Haën, Germany).

Histological sections of normal canine pancreas were used as positive controls to assess the specificity of the reactions. As a negative control, an isotype-matched antibody of irrelevant specificity (NeoMarkers, Fremont, CA, USA) was used in place of the primary antibody.

The slides were first evaluated at low magnification, assessing the sites with a higher concentration of positive cells. These sites were then digitally captured at higher magnification (200X) with a digital capture system (Nikon DS-L3, Nikon Instruments S.p.A, Firenze, Italy) connected to a light microscope (Nikon Eclipse 55i, Nikon Instruments S.p.A, Firenze, Italy) and five fields per slide were stored.

Fields were considered adequate for evaluation if villi were sectioned longitudinally, and the images were captured in order to include as many epithelial cells as possible.

Through the Nikon DS-L3 digital software (Nikon Instruments S.p.A, Firenze, Italy) the cells positively labeled for CgA (i.e. dark brown color) were marked in red and counted; thereafter, the other enterocytes were marked in green, blue and white and counted (Figure 1).

The positively-labeled cells were then expressed as a percentage with respect to the total number of cells in the epithelial layer, which allowed a reliable comparison among fields with different cellularity.

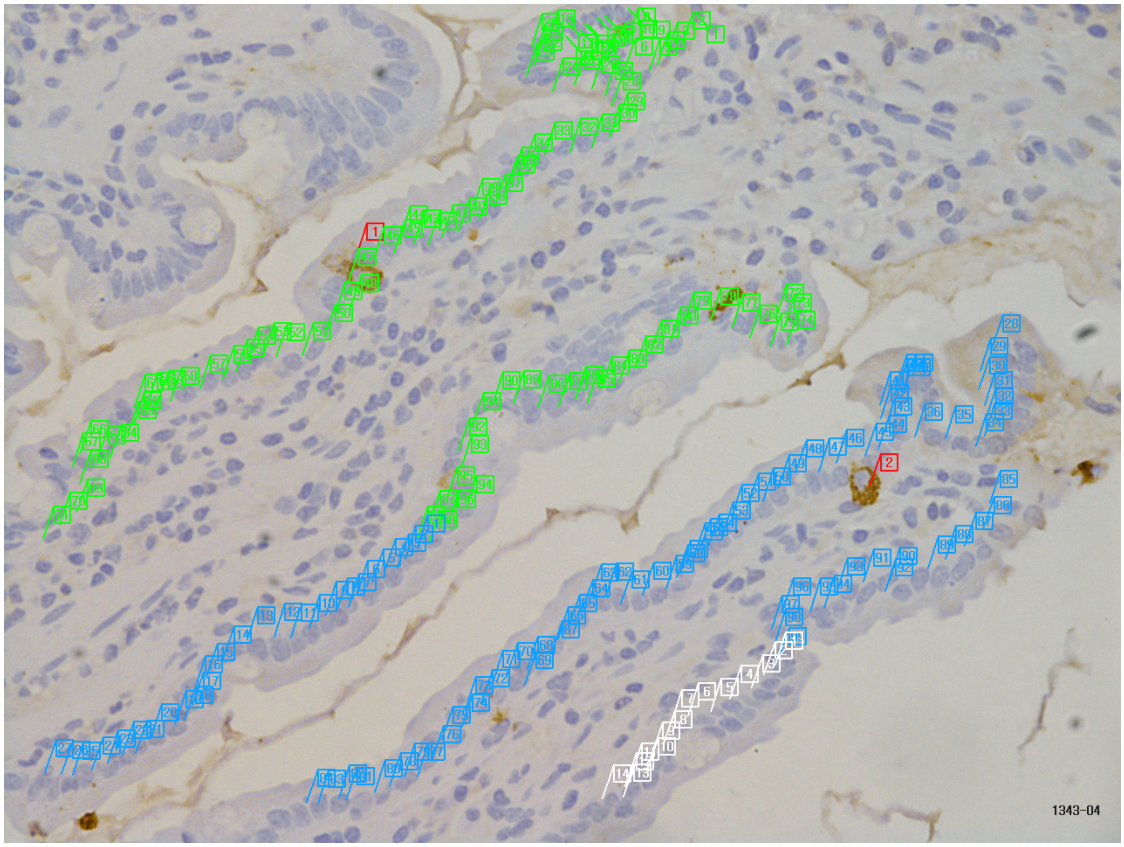

Figure 1: Exemplification of the system used to quantify the density of enterochromaffin cells in biopsy sections. In chromogranin-A immunostained sections, the positive labeled cells (enterochromaffin cells) are marked in red, and the enterocytes are marked in green, blue or white. The density of enterochromaffin cells is then expressed with respect to the enterocytes number, regardless of the area occupied by the inflammatory infiltrate. Streptavidin-biotin-peroxidase technique, hematoxylin counterstain, 200x.

STATISTICAL ANALYSIS

Statistical analysis was performed with a commercially available program (MedCalc sofware, Ostend, Belgium).
Assessment of data for normality was calculated by applying the D'Agostino-Pearson test.
Data were expressed as median (95% confidence interval).
A linear regression was applied between the percentage of duodenal EC cells and CCECAI, serum albumin, total cholesterol, serum folate, serum cobalamin, EASI, and histopathological index, respectively.
Values of $P < 0.05$ were considered significant.

RESULTS

CCECAI was performed in 19/21 dogs; albumin was analyzed in 20/21; total cholesterol in 18/21; serum folate in 16/21; serum cobalamin in 15/21 dogs; endoscopic score was calculated in 20/21; histopathological score in 18/21, and EC cell percentage with respect to duodenal epithelial layer cells in 14/21 dogs.
Median (95% confidence index) for CCECAI was 8 (6-10), for albumin was 1.59 g/dL (1.22-3.20), for serum cholesterol was 160 mg/dL (107-192), for serum folate was 8.09 µg/L (4.55-12.68), for serum cobalamin was 232 ng/L (159-271), for duodenal endoscopic score (EASI) was 1.5 (1-3.8), for duodenal histopathological score was 10.5 (8.4-13.2), and for duodenal EC cell percentage, with respect to duodenal epithelial layer cells, was 1.16% (0.94-1.98).
A significant negative relationship was evidenced only between duodenal EC cell percentage and serum folate (regression equation $y=16.89-6.14x$; coefficient of determination r^2: 0.7; significant level: P=0.007) (Table 1).
No significant relationship was evidenced between duodenal EC cell percentage and the other analyzed variables (Table 1).

Dependent Y	CCECAI	Albumin (g/dL)	Serum Cholesterol (mg/dL)	Serum folate (µg/L)	Serum cobalamin (ng/L)	EASI	Histopathological score
Independent X	EC cells (%)	EC cells (%)	EC cells (%)	EC cells (%)	EC cells (%)	EC cells (%)	EC cells (%)
R2	0.06	0.16	0.10	0.67	0.01	< 0.01	<0.01
Regression equation	Y=8.9999-0.8328x	Y=1.1174+0.7604x	Y=118.3226+22.5887x	Y=16.8928-6.1360x	Y=326.5332-36.5200x	Y=2.0598-0.09406x	Y=11.6543-0.3543x
F-ratio	0.6503	2.1287	1.0523	13.9130	0.098187	0.02837	0.05889
Significance Level	P=0.439	P=0.173	P=0.332	P=0.007	P=0.763	P=0.869	P=0.812

Table 1: Regression between the percentage of entherochromaffin cells (EC cells %) and clinical score (CCECAI), serum albumin, serum cholesterol, serum folate, serum cobalamin, endoscopic activity score index (EASI) and histopathological score, respectively.

DISCUSSION

One of the main results of the research is represented by the lack of relationship between duodenal EC cell concentration and the clinical condition, serum albumin, total cholesterol, serum cobalamin, endoscopic index, and histopathological score respectively.

Actually, this is not a surprising result. Even if each of the employed grading systems (CCECAI, clinico-pathological, EASI, histological) seems able to define the severity of canine IBD (9, 11, 12, 13), it is questionable if a significant association between, clinical signs, serum biomarkers and intestinal histopathological findings exists in canine IBD (14, 15).

With the exception of the recent paper by Bailey *et al.* (8), no previous studies have been performed on 5-HT metabolism in dogs with IBD. Moreover these researchers (8) evidenced a significant increase in 5-HT and CgA expression in duodenal biopsies of dogs with IBD with respect to healthy dogs, but no analysis was performed within the IBD group in relation to IBD severity.

On the other hand, it is not possible to compare our results about CgA duodenal expression with Bailey *et al's* study due to different cellular count criteria.

In fact, they (8) manually counted the number of cells stained positively for CgA in 20 random high power fields per slide and calculated average data, while we counted the CgA positive cells in five fields per slide digitally stored, then expressed it as a percentage with respect to the cellular total number of epithelial layers.

The only relationship that we have been able to identify, was the negative relationship between serum folate concentration and duodenal EC percentage, indicating the probable involvement of EC cells in the damage of proximal small bowel.

In fact, even if its sensitivity/specificity is not high, the decrease of serum folate could be read as an indication of proximal small intestinal malabsorption (16).

One of the limits of our study is that we counted the CgA positive cells, assuming, incorrectly, they all produce 5-HT, with the consequence of a possible 5-HT producing cells overcount.

Although CgA could mark all EC cells and not only the 5-HT producing EC cells, the duodenal CgA cell density has been elected in human gastroenterology as a reliable biomarker for the diagnosis of irritable bowel syndrome (7).

Furthermore, Bailey *et al*. (8) evidenced, in duodenal histologic samples examined from healthy dogs and dogs with IBD, a significant relationship between duodenal CgA positive cells and duodenal 5-HT producing EC cells.
In colonic biopsies of human beings, 50-60% of CgA positive cells are also positive for 5-HT (6).
We must also point out that the count of CgA cells instead 5-HT producing EC cells, in our study, involved the entire population, and poorly affects the results.
More important study bias is represented by the evaluation of the EC cell count omitting the 5-HT metabolic pathway, starting from tryptophan hydroxylase-1 (enzyme for 5-HT synthesis) up to 5-HT reuptake transport protein (SERT), expressed by platelets, nerve terminals, mucosal enterocytes, and vascular endothelial cells. These mediate the intracellular reuptake of 5-HT, reducing its availability (3, 4, 5).
In fact, an increase in mucosal 5-HT content could be due to a decrease of SERT level, rather than an hyperplasia of EC cells producing 5-HT (17).
The membrane expression of SERT can be altered by phosphorylation by Protein Kinase C (PKC) leading to internalization of SERT and reduction in 5-HT uptake rates in SERT-expressing cell lines.
Interferon-γ and tumour necrosis factor α significantly and synergistically impaired SERT functions, inducing a decrease in both SERT mRNA and protein levels (18).
Instead, IL-10 showed a dual effect on SERT, in relation with its concentration.
At a high concentration, IL-10 induced an increase of SERT activity and expression in the cell membrane, while a low concentration inhibited SERT activity (19).
In conclusion, further research is useful to advance the involvement of 5-HT in canine IBD pathogenesis, evaluating if SERT activity is related with IBD severity, and therefore if the decrease in 5-HT reuptake is linked to nociception and clinical signs in these patients.

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2. SIGNALEMENT: BREED, AGE AND SEX DISTRIBUTION

BREED:

The cause of CE in human beings and animal models is multifactorial and includes genetic and environmental components, as well as the mucosal immune system and intestinal microbiota, as described in previous chapters. In a recent meta-analysis of genome-wide association studies of Crohn's disease and ulcerative colitis several loci positively associated with disease were found, but further studies are needed to investigate heritability in human IBD and the other factors seems to be implicated in the complex genetic etiology of this disease.[1,2]

Moreover, the mode of inheritance of IBD in dogs not thoroughly understood, and may differ between dog breeds. A number of breed predispositions have been described in canine chronic GI disorders, thus strongly supporting a role for host genetics. Table 2.a resumes breed predisposition to chronic intestinal disease.

References	Breed	Findings
German et al, 2000; German et al, 2000(1); Craven et al, 2004; Allenspach et al, 2010; Kathrani et al, 2010; Dijkstra et al, 2010; Kathrani et al, 2011; Allenspach et al, 2016; Dandrieux, 2016 [3–11]	German Shepherd Dogs (GSD). Weimaraners, Rottweilers, Boxers and Border Collies. Golden Retrievers, West Highland white terriers (WHWT) and Labradors Retrievers	GSD appear to be predisposed to both idiopathic IBD and ARE (once called small intestinal bacterial overgrouth, SIBO). Possible described causes were: genetic predisposition, distinct expression pattern of TLR (increase in TLR4 and a very low expression of TLR5), dysbiosis and a heightened immune response within the intestinal mucosa. Other breeds are described to be highly susceptible to developing IBD in a retrospective paper in the south-eastern UK. Golden Retrievers, WHWT and Labradors Retrievers were other most commonly affected breeds in another retrospective study.
Lecoindre et al, 2010; Dijkstra et al, 2010; Dossin and Lauvé, 2011; Simmerson et al, 2014; Rudinsky et al, 2014; Bota et al, 2016[8,12–16]	Yorkshire Terriers Rottweiler	Rottweiler and Yorkshire terriers are susceptible to a potentially severe form of PLE. Clinical signs can be very mild to severe. Outcome is variable but in both breeds some dogs completely fail to respond to therapy, leading to a poor prognosis. On the contrary, one abstract reported retrospectively the effect of diet alone in Yorkshire terriers with PLE with complete resolution of clinical signs.
Wiberg et al, 1999; Clark et al, 2005; Batchelor et al, 2007; Mas et al, 2012; Westermarck et al, 2012; Alvarez et al, 2015; Evans 2015 [17–23]	German Shepherd Dogs, Rough-coated Collies, Chow Chows, and Cavalier King Charles Spaniels.	Exocrine pancreatic insufficiency (EPI) is a common condition in dogs, resulting from inadequate functional reserve of pancreatic acinar tissue. The most common cause of EPI in these breeds is pancreatic acinar atrophy. Clinical signs and maldigestion are usually not seen until 90% of the secretory capacity is lost. The endocrine function of the pancreas is usually spared in this process, but juvenile diabetes mellitus and concurrent EPI have been reported in the literature. Hereditability of EPI is demonstrated and is suggested to be a polygenic mode of inheritance.
Simpson et al, 2006; Craven et al, 2011; Manchester et al, 2013 [24–26]	Boxers, French Bulldogs	Granulomatous colitis is associated with selective intra-mucosal colonization by *Escherichia coli*, diagnosed by fluorescent in situ hybridization (FISH). Treatment of choice is fluorchinolone antimicrobials, but antimicrobial resistance has been demonstrated.
Ohmi et al, 2012; Igarashi et al, 2013; Igarashi et al, 2016[27–29]	Dachshunds	Miniature dachshunds are predisposed to develop inflammatory colorectal polyps (ICRPs), in Japan. Treatment option may be immunosuppressive therapy (prednisolone and cyclosporine). Dysbiosis is associated with ICRPs and it could be a potential therapeutic target. Two previously described dachshunds with ICRPs developed polypoid adenomas after a long-term course of the disease.

References	Breed	Findings
Hall and Batt, 1991; Polvi et al, 1998; Gardern et al, 2000[30–32]	Irish setter	Irish Setter dogs are predisposed to CE related to gluten sensitivity enteropathy (GSE). Underlying permeability abnormality may be involved in the pathogenesis of this enteropathy. Genetic transmission of gluten-sensitive enteropathy is under the control of a single major autosomal recessive locus.
Fyfe et al, 1991; Battersby et al, 2005; Grützner et al, 2009; Bishop et al, 2011; Grützner et al, 2013[33–37]	Chinese Shar Peis, Giant Schnauzer dog, Border collies, Australian shepherd dog	Chinese Shar Pei has a high prevalence of cobalamin deficiency compared to other breeds and healthy controls, and it can be subclinical. It is associated with mutation on chromosome 13 and could be an inheritable disorder. Several Shar-Peis with cobalamin deficiency have an alteration in serum homocysteine and methylmalonic acid compared to other breeds, suggesting that the function of the two intracellular cobalamin-dependent enzymes is impaired. Inherited selective intestinal malabsorption of cobalamin was observed in a family of Giant Schnauzer dogs and a simple autosomal recessive inheritance has been demonstrated. Border collies and Australian shepherd dogs in the USA have been reported to be affected by this disease.
Breitschwerdt et al, 1984; MacLachlan, et al 1988[38,39]	Basenji	A specific gastrointestinal disease (gastric rugal hypertrophy and lymphocytic plasmatic enteritis [LPE]) occurs in Basenji dogs and intestinal function was abnormal in both affected and asymptomatic Basenji dogs.
Littman et al, 2000; Vaden et al, 2000[40,41]	Soft Coated Wheaten Terriers (SCWT)	SCWT are predisposed to develop PLE and protein-losing nephropathy (PLN) and distinctive familial predisposition were demonstrated. Food allergies are present in SCWTs affected with PLE and/or PLN in an early phase of the disease process.
Ohno et al, 2006; Ohmi et al, 2011; Okanishi et al, 2013[42–44]	Shiba dogs	Shiba dog appears to be predisposed to chronic enteropathy with LPE and shows severe duodenal lesions and poor prognosis.
Berghoff et al, 2007; Qvigstad et al, 2008; Metzger et al, 2016 [45–47]	Norwegian Lundehunds	Lundehund syndrome (LS) is a severe disease affecting gastro-enteric system characterized by atrophic gastritis and PLE, which led to predisposition to the development of gastric neoplasia. Approximately 50% of all Norwegian Lundehund living in North America are affected. A genetic predisposition has been investigated but further studies are needed to understand the underlying complex genetic mechanisms.

Table 2.a: Breed predisposition to common chronic gastrointestinal disease in dogs.
IBD, inflammatory bowel disease; ARE, antibiotic responsive enteropathies; TLR, toll-like receptors; PLE, protein loosing enteropathies; LPE, lymphocytic-plasmacytic enteritis

AGE

Although there is no definitively age predilection documented for CE, ARE and FRE phenotypes appear to be more common in younger dogs, with respect to SRE.[10,11,48–50] ARF is very common in dogs younger than 1 year of age[51], although in many studies the average age of the FRE dogs is higher than 1 year.[11,48,52] ARE, as well as tylosin responsive enteropathy typically affects middle-aged, large-breed dogs.[11,53,54] To the contrary, middle-aged to older dogs are more prone to have SRE or PLE,[9–11,44,48,55] even if in some breeds it can occur even in younger individuals.[12,40] Although the age of presentation can help distinguish CE phenotype, in clinical setting, it is important to remind that FRD, ARE or SRD can occur at any age.[11]

SEX

No definite sex predisposition is reported in dogs with CE.[7,56] Even if in some breeds (Yorkshire terrier with PLE[12] and SCWT dogs[40]) a female predisposition was described. The is not unanimous on this aspect, as in another study more males than females were affected by CE.[9] Moreover, a recent study has demonstrated that neutering is associated with increased risk for certain autoimmune disorders, included IBD.[57]

Female predisposition is well established in autoimmune disorders in human beings[58,59] and in other acquired autoimmune diseases in dogs. [60–63] Although in contrast to other female-specific autoimmune diseases, female predominance in IBD is not a general feature in human beings, and when present, it is subject to great geographical variation. Geographical differences in the female:male ratio observed across different IBD populations might be explained with differences in the exposure to environmental factors associated with the disease (infectious agents, use of oral contraceptives, antibiotics use, smoking).[64]

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2.1 BREED AND SEX PREDISPOSITION IN DOGS WITH CHRONIC ENTEROPATHIES: RETROSPECTIVE STUDY FROM 2010 TO 2015

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SUMMARY

Many breeds are predisposed to develop CE and many breed-specific related enteropathies have been described in literature, and no definitive sex predisposition have been demonstrated. None of the studies available in literature were performed in Italy, and therefore there is no information regarding breed or sex predisposition in the Italian canine population.

The aim of this study was to evaluate the sex or breed predispositions in dogs with CE presented to the Veterinary Teaching Hospital of University of Bologna between January 2010 and December 2015.

Dogs of any sex, breed, and age were included if a minimum workup (hematology, plasma biochemistry profile, and fecal parasitology) had been performed and if a final diagnosis of CE, with no concomitant disorders, was recorded (n=268 dogs). A control population was included to evaluate sex and breed predispositions in CE. The control population included all dogs admitted to the hospital in the same period of time (n=33130 dogs). Odds ratios and Chi Square tests were used to analyze breed and sex predispositions, respectively, significance was set at p=0.05.

In dogs diagnosed with CE the following phenotypes were diagnosed: FRE 27.9% (75/268), ARE 20.8% (56/268), IBD 20.8% (56/268), PLE 13.4% (36/268), and HUC 1.1% (3/268). In 15.6% (42/268) the exact phenotype could not be determined.

Breeds predisposed to CE and each phenotype are reported in Table 1. Male dogs were shown to be predisposed to develop CE, in particular IBD (Table 2).

Results of this study confirm a predisposition of certain breeds to develop CE, such as GSD,[1–4] Boxer,[4.5] Yorkshire Terrier,[6.7] and Border Collie.[4] Other breeds that were not previously reported include: French Bulldog, Miniature Puddle, Pincher, Jack Russel Terrier and Siberian Husky. Moreover, two Italian breeds were shown to be predisposed to CE: Maltese and Bolognese. The results of this study also demonstrate a male predisposition to IBD in the population of interest. Female predisposition is well established in autoimmune disorders in human beings[8.9] and in other acquired autoimmune disease in dogs. [10–13] Female predisposition in CE in humans is not demonstrated and it is subject to great geographical variation due to differences in the exposure to environmental factors associated with the disease.[14]

In conclusion, this study confirms that CE is more frequent in pure breed dogs and certain breeds are predisposed. Moreover, this is the first study that reports a male predisposition to IBD in dogs.

Breed	Control population	CE dogs	OR	95% CI	z statistic	P-value
			CE			
Mixed breed	10875	54	1			
GSD	2087	25	2.4295	1.5087 to 3.9125	3.652	P = 0.0003
Boxer	800	15	3.8291	2.1511 to 6.8159	4.564	P < 0.0001
Miniature Poodle	882	11	2.5307	1.3185 to 4.8576	2.791	P = 0.0053
Maltese	710	9	2.5728	1.2651 to 5.2319	2.609	P = 0.0091
Yorkshire Terrier	665	9	2.7492	1.3516 to 5.5923	2.792	P = 0.0052
Pinscher	713	8	2.2739	1.078 to 4.7967	2.157	P = 0.0310
French Bulldog	381	8	4.2979	2.0309 to 9.0956	3.812	P = 0.0001
Jack Russel Terrier	540	7	2.6317	1.1918 to 5.8113	2.394	P = 0.0167
Border Collie	346	6	3.5363	1.511 to 8.2764	2.911	P = 0.0036
Bolognese	362	5	2.8066	1.1159 to 7.0585	2.193	P = 0.0283
Siberian Husky	161	3	3.8049	1.1772 to 12.2982	2.232	P = 0.0256
			FRE			
Mixed breed	10875	20	1			
Border Collie	346	3	4.7471	1.4039 to 16.0514	2.506	P = 0.0122
			ARE			
Mixed breed	10875	11	1			
Dachshund	675	3	4.4091	1.2271 to 15.8421	2.274	P = 0.0230
Yorkshire Terrier	665	3	4.4757	1.2456 to 16.0819	2.297	P = 0.0216
			IBD			
Mixed breed	10875	11	1			
GSD	2087	6	2.8476	1.0519 to 7.7084	2.06	P = 0.0394
Boxer	800	5	6.2115	2.1529 to 17.9216	3.378	P = 0.0007
Maltese	710	3	4.1908	1.1665 to 15.0561	2.196	P = 0.0281
			PLE			
Mixed breed	10875	7	1			
GSD	2087	5	3.7286	1.1822 to 11.7592	2.246	P = 0.0247
Rottweiler	372	3	12.6225	3.2511 to 49.0081	3.664	P = 0.0002

Table 1: Breeds predisposed to CE and each phenotype. Data are presented on the OR of developing CE compared with the mixed-breed dog. together with the 95% CI z- statistic and *p*-value .
CE, chronic enteropathies; FRE, food-responsive enteropathies; ARE, antibiotic responsive enteropathies; IBD, inflammatory bowel disease; PLE, protein loosing enteropathies; CI, Confidence interval; GSD, German shepherd dog; OR, Odds ratio.

	FRE	ARE	IBD	PLE	All CE	Control population
Male	41 (54.7%)	26 (46.4%)	44 (78.6%)	21 (58.3%)	160 (59.7%)	16654 (50.3%)
Female	34 (45.3%)	30 (53.6%)	12 (21.4%)	15 (41.7%)	108 (40.3%)	16476 (49.7%)
***p*-value**	**0.4467**	**0.5658**	**< 0.0001**	**0.3334**	**0.0021**	

Table 2: Sex predisposition to CE and each phenotype.

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3. CLINICAL SIGNS

Clinical signs of dogs with CE can vary from mild or subclinical to severe with life threating GI signs. The severity of clinical signs (such as diarrhea, vomiting, or weight loss) has not been reported to predict response to treatment or prognosis, and neither is related with the disease severity.[1] However clinical severity at the time of diagnosis was significantly different between phenotypes in some reports, with FRE having less severe clinical signs with respect to ARE or IRE.[2,3] Others symptoms, such as anorexia, severe weight loss, and ascites are associated with poor prognosis.[1,4]

Therefore in a clinical settings is important to recognize common mild or subclinical symptoms and be aware of the ones that can be less common, but more severe.

In the following section clinical signs of dogs with CE are briefly reviewed and divided into "common", "less common", and "uncommon" symptoms. Finally, available and widely used clinical scoring systems for dogs with CE are reported.

COMMON CLINICAL SIGNS:

DIARRHEA AND DEFECATION ABNORMALITIES

Diarrhea is an increase in the frequency, fluidity, or volume of feces that is best characterized by duration (acute versus chronic), pathophysiologic mechanism, and anatomic location.[5]

Diarrhea is the most common symptom in GI disorders, although it is important to remember that it may be absent.[6] Stool consistency, stool frequency, presence of blood or mucus, or alteration of defecation (such as tenesmus, dyschezia, urgency) allows clinicians to localize the GI problem (Table 3.a). Watery diarrhea is typical for small bowel disease and is commonly associated with weight loss and sometimes vomiting.[7] Large bowel diarrhea, on the other hand, characterized by increased frequency of defecation of small amounts of feces, often admixed with mucous and/or fresh blood (hematochezia), urgency, and tenesmus.[8–10] In one study[1] large bowel diarrhea is associated with FRE, although, in many occasions patients show signs of large and small bowel involvement, and therefore a diffuse GI disease is suspected.

Sign	Small Bowel	Large Bowel
Tenesmus	Rare	Common
Frequency	Normal to 2-3 times x day	>3 time x day
Urgency	Uncommon	Common
Volume	Increased	Decreased
Mucus	Rare	Common
Blood	Melena	Hematochezia
Steatorrehea	May be present	Absent
Dyschezia	Absent	Often present
Weight loss	Common	Uncommon
Vomiting	May be present	May be present

Table 3.a: Differentiation of small versus large bowel diarrhea based upon clinical signs and the physical appearance of feces.[5]

VOMITING

Vomiting is seen in canine CE although invariably accompanies and is less severe than diarrhea. However in cats, vomiting is often the predominant clinical sign of small intestinal IBD.[11]

Contents of vomit, association with food, signs of nausea, and appetite are necessary information to localize the disease (e.g. gastric, colonic, or esophageal disorders or systemic disease) and understand the cause of vomiting (e.g. inflammatory process rather than dysmotility).

Regurgitation is usually associated with esophageal disorders, but sometimes it can be present in CE as a consequence of dysmotility disorders.[12] Hematemesis is usually associated with more severe disease, which causes mucosal ulceration or erosion.

Bilious vomiting syndrome in dogs is a relatively common occurrence, and it is thought to result from a reflux of duodenal fluid into the gastric lumen causing mucosal irritation.[13] Inflammation of GI tract and motility disorders can predispose dogs to this syndrome. Dogs with bilious vomiting syndrome will tend to vomit a yellow-colored bile vomit when they are fasted.[13]

WEIGHT LOSS

Undernutrition, also called malnutrition, is a cause of weight loss in GI disorders (Figure 3.a). Malnutrition can lead to cachexia with severe consequences for the patient (Table 3.b).[14] In one study, severe (≥30%) weight loss was a negative prognostic factor.[4]

GI, pancreatic, and hepatobiliary disorders are common causes of malnutrition in dogs. Pathophysiologic mechanisms include decreased food intake (e.g. decreased appetite), maldigestion, malabsorption, inflammation, increased nutritional requirements, and drug–nutrient interactions.[14]

Weight loss is a typical finding in small bowel disease, but can be also found in large bowel disease.

Complications of malnutrition	
Compromised wound healing	Decreased gastrointestinal function
Immune suppression	Decreased pancreatic function
Impaired muscle strength	Decreased water and sodium excretion
Fatigue	Increased tendency to develop edema
Poor thermoregulation	Increased morbidity and risk of secondary diseases
Decreased respiratory function	Increased risk of death

Table 3.b: Complication of malnutrition[15]

Figure 3.a: GSD with CE showing evident weight loss

APPETITE CHANGES

Appetite changes can be variable in CE, with some cases demonstrating polyphagia, others showing differing severities of anorexia, but most cases displaying no appetite changes. One study revealed that a decrease in appetite is a negative prognostic factor in dogs with lymphocytic plasmacitic enteritis (LPE).[4]

Pica (ingestion of non-nutritive items), ingestion of foreign bodies, and abnormal chewing behaviors can be common in dogs with CE in author's experience. It is widely accepted that those actions are consequences of behavior and GI disorders, but there are very few studies (or anecdotal reports) focusing on the relationship of pica and abnormal chewing behaviors in dogs or cats with CE.[16–18]

LESS COMMON CLINICAL SIGNS

ASCITES, PLEURAL EFFUSION, AND PERIPHERAL EDEMAS

Ascites is defined as fluid accumulation within the peritoneal cavity. Ascites is usually associated with PLE, and the cause of its formation is the decrease in oncotic pressure when serum albumin concentration falls below 1.5 g/l. In PLE usually fluid is pure or modified transudates, but also chylous ascites has been reported.[19] Presence of ascites in PLE dogs varies from 18%[20] to 100%.[1] Pleural effusion can be associated with ascites and appears to be common in Yorkshire Terriers with PLE and it can be the only clinical sign on presentantion.[6,21] Peripheral edema, such as pitting edema of the limbs, scrotum or face, can be present.[19] In author's experience it seems less common, but no studies evaluate the incidence of this clinical sign (Figure 3.b).

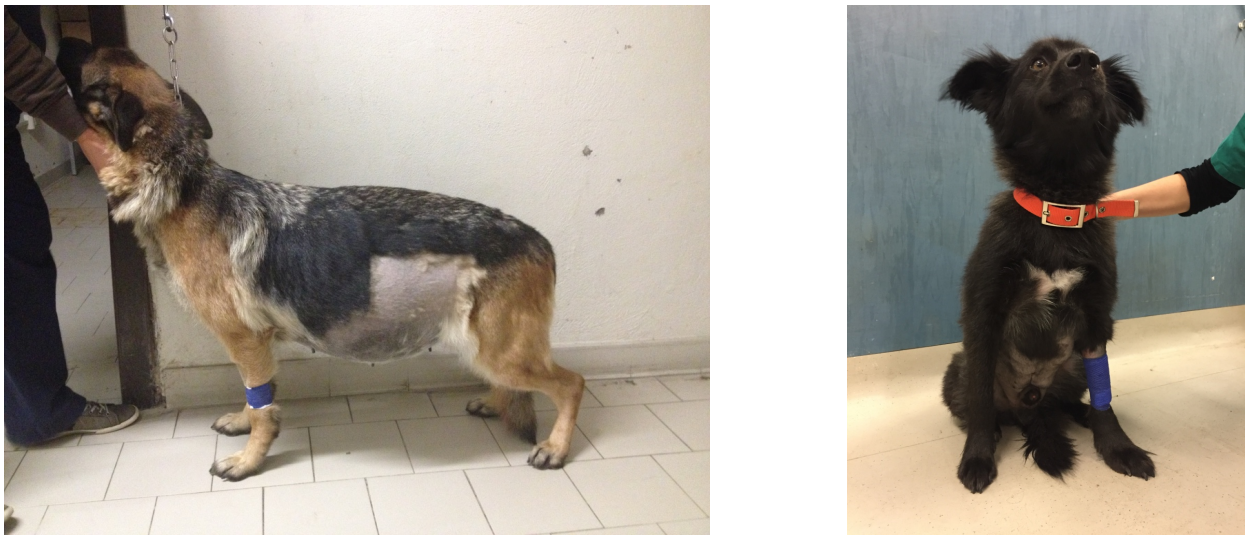


Figure 3.b: Two dogs with PLE. The picture on the left shows a GSD with severe ascites. Picture on the right shows a mixed breed dog with pleural and abdominal effusion and subcutaneous edema of ventral abdomen.

DERMATOLOGICAL ABNORMALITIES

Dermatological signs are characteristic in ARF. The most common symptom of ARF is non-seasonal pruritus.[22] Pruritus can be either generalized or limited to face, ears, paws, axillae, inguinal, or perineal region.[23] Other signs include papules, erythema excoriations, epidermal collarettes, hyperpigmentation, pododermatitis, and seborrhea. Otitis *externa* is an important indication for ARF and it may be the only sign. Finally, some dogs can only show recurrent bacterial pyoderma (with or without pruritus).[23]

In one study, FRE dogs showed pruritus more commonly compared with other disease phenotypes (ARE or IBD).[1] Presence of concurrent dermatological signs in a dog presented for chronic GI symptoms could potentially be indicative of FRE.[22] The connection between dermatologic and GI symptoms is not definitely indicative for FRE and no studies evaluated this correlation.[22,23]

UNCOMMON CLINICAL SIGNS

THROMBOSIS

In PLE a hypercoagulable state is demonstrated as a consequence of reduced antithrombin III (AT III) plasma concentration, increased thrombin-antithrombin complexes, or an abnormal thromboelastogram.[24–26] Therefore thromboembolic events in association with PLE have been reported in several studies,[19] with incidence ranging from 12-18%[27] to 7.5%,[28] respectively. Aortic, femoral artery, and pulmonary thrombosis with sudden death has been reported.[29–32]

HYPOCALCEMIA- RELATED SYMPTOMS

Hypocalcemia is a common finding in dogs with PLE.[33,34] Usually no clinical signs related to hypocalcemia are evident, even with very low ionized calcium. In other reports, hypocalcemia was associated with radiographic evidence of osteopenia alone[35], or it induced twitching episodes or seizures in dogs with PLE.[34,36,37]

CLINICAL SCORING SYSTEMS

Clinical indices remain the most widely used tools for assessing disease activity in human and veterinary CE patients.[38] Scoring indexes can be used to assess disease severity,[1,39] correlate with prognosis,[4] and can be also used in research setting.

Commonly used clinical score indexes are briefly described in the following section.

CIBDAI AND CCECAI

Clinical IBD activity index and Canine CE clinical activity index were developed and validated several years ago.[1,39] Today CIBDAI and CCECAI are widely accepted and used in research and clinical settings. CIBDAI evaluates only clinical signs, whereas CCECAI integrates biochemical parameters (serum albumin concentration) (Table 3.c).

BODY CONDITION SCORE (BCS) AND MUSCLE CONDITION SCORE (MCS)

Assessment general wellness and physical condition are important as CE has a huge impact on weight (body fat and muscle mass). Using a consistent method and scale, like body condition score (BCS) and muscle condition score (MCS), can assist clinicians in making a correct diagnosis, recommending dietary support, and identifying changes over time.[40] Moreover, in research it allows a prompt understanding of dog's general state of nutrition better than body weight (BW) alone because different dog sizes exist.

The BCS 9 point scale is widely used in veterinary practices, although a limited number of studies in dogs and cats evaluate its usefulness (Table 3.d).[41]

Evaluation of MCS includes visual examination and palpation over the temporal bones, scapulae, lumbar vertebrae and pelvic bones in order to appreciate the muscle mass that cover those bony prominences. A simple MCS scale is currently under development and validation.[40]

FECAL SCORE

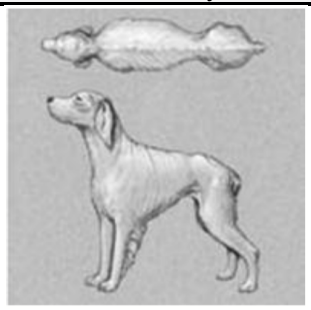
Examination of characteristics of defecation (attitude and frequency), along with fecal consistency provides insight into the function of the intestinal tract. Fecal consistency evaluates the amount of moisture in the stool and can be used for diagnosis of GI disorders and assessing improvement. Many fecal scoring (FS) systems have been used in veterinary literature, but none have been correctly validated. The Purina 7 point fecal score is an easy and widely used scoring system in current literature.[42] Ideally, in a healthy animal, stools should be firm but not hard, pliable and segmented, and easy to pick up (Score 2-3) (Table 3.e).

CIBDAI Canine inflammatory bowel disease activity index	CCECAI Canine chronic enteropathy clinical activity index
Attitude/activity 0 normal 1 slightly decreased 2 moderately decreased 3 severely decreased	Attitude/activity 0 normal 1 slightly decreased 2 moderately decreased 3 severely decreased
Appetite 0 normal 1 slightly decreased 2 moderately decreased 3 severely decreased	Appetite 0 normal 1 slightly decreased 2 moderately decreased 3 severely decreased
Vomiting 0 normal 1 mild (1 x per week) 2 moderate (2–3 x week) 3 severe (>3 x week)	Vomiting 0 normal 1 mild (1 x per week) 2 moderate (2–3 x week) 3 severe (>3 x week)
Stool consistency 0 normal 1 slightly soft feces 2 very soft feces 3 watery diarrhea	Stool consistency 0 normal 1 slightly soft feces 2 very soft feces 3 watery diarrhea
Stool frequency 0 normal 1 slightly increased (2–3 x day) or fecal blood, mucus or both 2 moderately increased (4–5 x day) 3 severely increased (>5 x day)	Stool frequency 0 normal 1 slightly increased (2–3 x day) or fecal blood, mucus or both 2 moderately increased (4–5 x day) 3 severely increased (>5 x day)
Weight loss 0 none 1 mild (5%) 2 moderate (5–10%) 3 severe (>10%)	Weight loss 0 none 1 mild (5%) 2 moderate (5–10%) 3 severe (>10%)
Total: 0 - 3: clinically insignificant 4 – 5: mild 6 – 8: moderate ≥ 9: severe	Albumin levels 0 albumin >20g/L 1 albumin 15–19.9 g/L 2 albumin 12–14.9 g/L 3 albumin <12 g/L
	Ascites and peripheral edema 0 none 1 mild ascites or peripheral edema 2 moderate amount of ascites/ peripheral edema 3 severe ascites/pleural effusion and peripheral edema
	Pruritus 0 no pruritus 1 occasional episodes of itching 2 regular episodes of itching, but stops when dog is asleep 3 dog regularly wakes up because of itching
	Total: 0 - 4 Clinically insignificant 5 - 6 Mild 7 - 9 Moderate 10 - 12 Severe >12 Very severe

Table 3.c: CIBDAI[39] and CCECAI[1] score for dogs

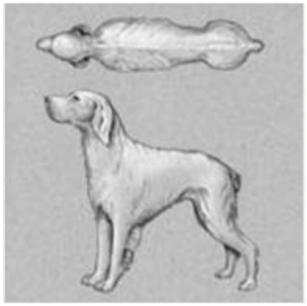
BCS
Body condition score, 9 point scale

BCS1
Ribs, lumbar vertebrae, pelvic bones and all bony prominences evident from a distance. No discernible body fat. Obvious loss of muscle mass.



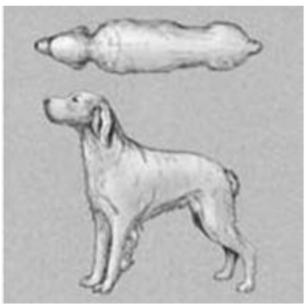
BCS 2
Ribs, lumbar vertebrae and pelvic bones easily visible. No palpable fat. Some evidence of other bony prominence. Minimal loss of muscle mass.

BCS 3
Ribs easily palpated and may be visible with no palpable fat. Tops of lumbar vertebrae visible. Pelvic bones becoming prominent. Obvious waist and abdominal tuck.



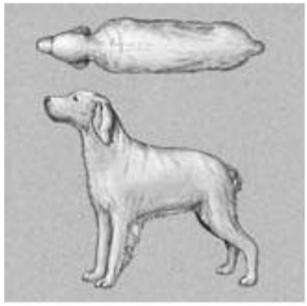
BCS 4
Ribs easily palpable, with minimal fat covering. Waist easily noted, viewed from above. Abdominal tuck evident.

BCS 5
Ribs palpable without excess fat covering. Waist observed behind ribs when viewed from above. Abdomen tucked up when viewed from side.



BCS 6
Ribs palpable with slight excess fat covering. Waist is discernible viewed from above but is not prominent. Abdominal tuck apparent.

BCS 7
Ribs palpable with difficulty; heavy fat cover. Noticeable fat deposits over lumbar area and base of tail. Waist absent or barely visible. Abdominal tuck may be present.



BCS 8
Ribs not palpable under very heavy fat cover, or palpable only with significant pressure. Heavy fat deposits over lumbar area and base of tail. Waist absent. No abdominal tuck. Obvious abdominal distention may be present.

BCS 9
Massive fat deposits over thorax, spine and base of tail. Waist and abdominal tuck absent. Fat deposits on neck and limbs. Obvious abdominal distention.

Table 3.d: BCS score for dogs. BCS of 4 and 5 are considered normal.
Body Condition Score (BCS) chart, Ralston Purina Company, St Louis, Mo

Fecal Score 7 point scale			
FS1 Very hard and dry; often expelled as individual pellets; requires much effort to expel from body; no residue left on ground when picked up.		**FS 5** Very moist, but has a distinct shape; piles rather than distinct logs; leaves residue and loses form when picked up.	
FS 2 Firm, but not hard; pliable; segmented in appearance; little or no residue on ground when picked up.		**FS 6** Has texture, but no defined shape; present as piles or spots; leaves residue when picked up.	
FS 3 Log-shaped; little or no visible segmentation; moist surface; leaves residue on ground, but holds form when picked up.		**FS 7** Watery; no texture; flat puddles.	
FS 4 Very moist, soggy; log-shaped; leaves residue and loses form when picked up.			

Table 3.e: Fecal score for dogs. Fecal score of 2 and 3 are considered normal.
Nestlé Purina Fecal Scoring (FS) System

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3.1 FINAL DIAGNOSES IN DOGS WITH CHRONIC VOMITING AND/OR DIARRHEA: RETROSPECTIVE STUDY FROM 2010 TO 2015

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Student thesis, year 2016

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SUMMARY

Chronic diarrhea and chronic vomiting are frequent complaints in clinical setting and are the clinical presentations of many diseases (e.g. gastrointestinal, neoplastic, metabolic disorders, and others causes).[1–3]
The aim of this study was to evaluate the final diagnoses and determine the prevalence of different diseases in dogs presented for chronic diarrhea and/or vomiting.
Medical records of 608 dogs presented to the Veterinary Teaching Hospital of University of Bologna between January 2010 and December 2015 with chronic diarrhea, vomiting, or both were retrospectively reviewed. Dogs of any sex, breed, and age were included if a minimum workup (hematology, plasma biochemistry profile, and fecal parasitology) had been performed and if a final diagnosis was recorded (463/608).
A diagnosis of CE was determined in 69.7% (323/463) and included: FRE 25.7% (83/323), ARE 19.8% (64/323), IBD 21.3% (69/323), PLE 11.8% (38/323), HUC 0.9% (3/323). In 20.4% (66/323) of dogs with CE the exact phenotype could not be determined. In CE group, 83% (268/323) of dogs had only CE and 17% (55/323) had a concomitant disorders that were potentially a cause of chronic vomiting and/or diarrhea.
Another GI disorder cause of vomiting and/or diarreah was diagnosed in 18.3% (85/463) of dogs. Of those, 41% (35/85) had hepatic or pancreatic disorders (including EPI, chronic hepatitis and chronic pancreatitis), 21% (18/85) had intestinal neoplasia, 26% (22/85) infectious GI disease (bacterial and parasitic), 12% (10/85) mechanical/obstructive causes (included 2 dogs with short bowel syndrome [SBS] and 1 dog with chronic intestinal pseudo obstruction [CIPO]).
A metabolic cause of chronic vomiting and/or diarrhea was diagnosed in 11.8% (55/463) of dogs and included 43.6% (24/55) chronic nephropathy, 34.5% (19/55) endocrine diseases, 18.1% (10/55) extra GI neoplasia, and 1.8% (1/55) porto-systemic shunt (PSS).
Chronic diarrhea was found to be the predominant symptom in CE dogs, while vomiting alone and vomiting and diarrhea was more frequent in dogs with metabolic disorders ($p<0.0001$).
In conclusion, similar to a previous study[1] CE was the most frequent cause of chronic GI of dogs in our population, followed by other causes of GI disease and metabolic disorders. Finally, chronic diarrhea was more frequent in dogs with CE, while chronic vomiting was more common in metabolic disorders.

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4. DIAGNOSIS

Diagnosis of CE is based on the recognition of clinical signs, exclusion of other causes of chronic GI signs, assessment of severity (e.g. loss of proteins, signs of malabsorption, coagulation abnormalities, or electrolyte imbalance), and identification of intestinal inflammation. The different phenotypes FRE, ARE, IRE, or NRE are subsequently diagnosed based on the response to treatment. In terms of frequency, FRE is generally more common in respect to other phenotypes and PLE is the least frequent (Figure 4.a).[1,2]
In this section common and newly discovered diagnostic tools for CE in are quickly reviewed.

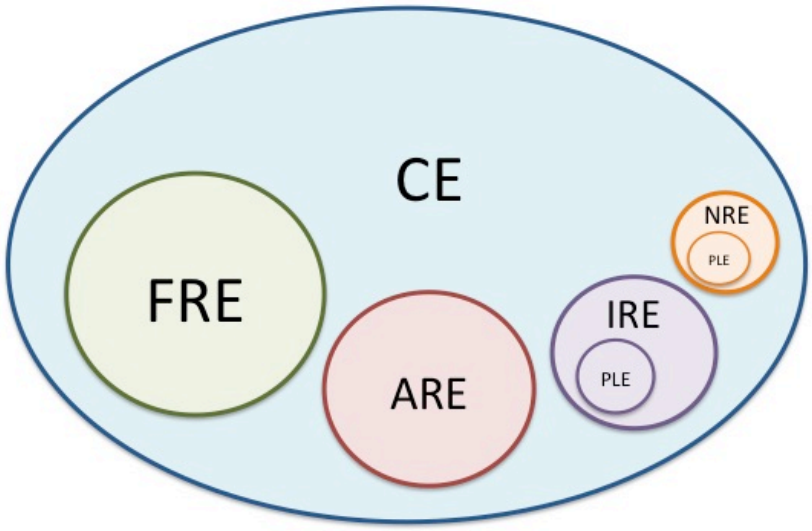

Figure 4.a: Among CE, FRE phenotype is the most frequent followed by ARE, IRE, and NRE. PLE is usually IRE and is still less frequent.
CE, chronic enteropathies; FRE, food responsive enteropathy; ARE, antibiotic responsive enteropathy; IRE, immunosuppressant responsive enteropathy; NRE, non responsive enteropathy; PLE, protein-losing enteropathy.

BASIC WORK-UP

FECAL PARASITOLOGY

GI parasites are a common cause of chronic GI disorders in dogs. A routine fecal examination for parasites should always be included in the early diagnostic workup of an animal with GI disease.[3,4] Independent of the results of fecal parasitology, a treatment with broad spectrum anthelmintics drugs (e.g. fenbendazole) is always recommended.
Fecal examination techniques include:[3]

- Direct fecal smears; to visualize motile trophozoites (e.g. *Giardia*)
- Fecal flotation, with or without centrifugation; allows eggs, oocysts and cysts, to be identified
- Antigen testing and PCR assays; mainly used for *Giardia*, *Tritricomonas* (cat), *Cryptosporidium* spp., and canine Schistosomiasis.

Fecal shedding of GI parasites can be intermittent and some clinically normal animals can shed parasites without showing clinical signs. On the other hand, a positive diagnostic test does not prove that the parasite is causing disease.[4] Therefore, a repeated fecal panel and collection of 2-3 day of feces, increases the sensitivity of the exam.

Giardia spp. is a common zoonotic[5] GI parasite that can cause gastroenteritis and can be transmitted directly via contaminated water, food, or physical contact.[6,7] Recognition of this infection is important for a correct diagnosis of CE and its zoonotic potential. Prevalence in dogs appears to be high, but it important to remember that commonly used methods for *Giardia* identification can have many false positive results.[3,8] Recurrence of *Giardia* infection seems also a common feature in CE, although no studies are available to asses this connection.

BLOOD WORK AND URINALYSIS

Most of the time, hematology presents no typical alterations. Signs of hemoconcetration or leukocytosis can be seen in dogs with CE and are non-specific. Biochemistry panel had a crucial role in understanding the presence of other disease (e.g. electrolyte inversion in Addison, increase in liver enzymes in chronic hepatitis, or creatinine in chronic kidney disease) and detect the severity of of malabsorption.
Table 4.a reports principal clinicopathological abnormalities, proposed pathophysiological mechanism, and the importance for the patient.

Variable	Alteration	Comment
Neutrophils	Increased	Neutrophilia with or without left shift can occur.[9,10]
Eosinophil	Increased	Eosinophilia is sometimes present[11] and it is associated with allergy, parasitism, and eosinophilic enteritis (EE).[9] In SCWT, eosinophilia is present approximately in 21% of dogs and could be an early marker for SCWT protein-losing diseases.[12]
Lymphocytes	Decreased	Lymphopenia is present in PLE dogs as a consequence of GI loss due to lymphangectasia.[10,13] In SCWT, it is present in approximately 37% of dogs.[12]
Platelets	Increased or decreased	Thrombocytosis and thrombocytopenia can be both present in CE. The first one is well defined in human patients with IBD, is proportional to the activity of the disease,[14–16] and is associated with iron deficiency.[17,18] In dogs it is reported in Yorkshire,[10] and other dogs,[9,11] although thrombocytopenia appears to be more common in dogs with CE.[11]
PCV and RBC	Increased or decreased	Dehydration, due to water loss and decreased intake, can led to an increase in PCV and RBC counts. Anemia can be also present, ranging from 12 to 18% in some reports, and it has many causes.[11,19–21] Microcytic, hypochromic anemia can be consequence of a decrease iron absorption. Mild non-regenerative anemia can be present as consequence of chronic inflammation.[21] Normocytic, normochromic anemia may be associated with GI bleeding, and it can be regenerative or non-regenerative, depending upon chronicity of the condition.[22] Finally, less frequently erythroblastic anemia can be present due to cobalamin deficiency.[23]
Total protein **Albumin**	Decreased	Panhypoproteinemia is characteristic of PLE, and it is defined as albumin concentration is less than 2g/l with normal albumin:protein ratio.[1,2,13,24] The pathophysiology of hypoprotidemia may involve reduced appetite, malabsorption due to a reduction in intestinal surface area, such as with villus atrophy or fibrosis, haemorrhage or exudation of protein into the gastrointestinal tract, and increased intestinal permeability.[11] Mild hypoalbuminemia in IBD dogs also occur,[13] and in both IBD and PLE it increases during treatment. Urinalysis with urinary protein:creatinine (UPC) ratio should always be performed in presence of decreased albumin in order to exclude PLN.
Cholesterol	Decreased	Hypocholesterolemia can be frequently observed in dogs with CE and PLE. A decrease in cholesterol is caused by lymphangiectasia and malabsorption.[20,25]
Liver enzymes (ALT, AST, SAP)	Increased	Liver enzymes may be elevated secondarily in GI disease because of portal venous transport of toxins and/or bacteria from a compromised intestine, but overall liver function (as assessed by serum bile acids) will usually be normal.[25]

Variable	Alteration	Comment
Iron profile	Modified	Iron deficiency mainly results from chronic blood loss in the intestine, but iron malabsorption due to inflammatory activity may also contribute. In human medicine, it is well described that iron deficiency can cause anemia and thrombocytosis.[17,18] Modifications in serum iron profile of CE dogs, along with anemia, have been reported in one study.[21] Iron profile shows iron values at the lower end of the reference range, normal ferritin, normal or slightly low total iron binding capacity (TIBC), and high CRP concentration support the hypothesis that anemia was due to chronic disease.
C Reactive Protein (CRP)	Increased	CRP is commonly increased in dogs with CE. In some reports it seems to correlate with clinical activity score,[1] but not in others.[26–28] In one study, CRP appears to be higher in dogs with PLE compared to FRE although it was not indicative of outcome or survival time.[24] Although, for other authors, it is useful to asses severity of the disease and monitor the treatment response.[29,30]
Canine specific pancreatic lipase immunoreactivity (cPLI)	Increased	Serum cPLI concentration is highly specific for exocrine pancreatic function, and is the gold-standard for diagnosis of pancreatitis in dogs.[31] In one study a proportion of dogs with IBD had high cPLI concentrations and it was correlated with a poor outcome.[32] Based on this study, measurement of serum PLI concentrations may be warranted in dogs and cats with IBD, as animals with IBD and high serum PLI concentrations may require more extensive work-up and aggressive management.[33]
Total Calcium and ionized calcium	Decreased	Calcium is found in three forms within plasma; the physiologically active ionized form accounts for about 50%, the complexes formed with lactate, citrate, and bicarbonate accounts for about 10%, and the protein bound form about 40%.[34] Measurement of total calcium is mostly influenced by albumin concentration, phosphate, and acid-base status, therefore assessment of ionized calcium is a much better indicator of calcium homeostasis. Ionized hypocalcemia is commonly reported in CE especially in PLE.[4,10,35,36] Supplementation of calcium is suggested because clinical signs associated with hypocalcemia, such tremors, seizures and osteopenia as been reported.[10,35–38]
Magnesium	Decreased	Low magnesium concentration is frequently present in dogs with CE especially the ones with PLE, and it is frequently associated with hypocalcemia.[10,35,37] Supplementation of magnesium is warranted to reestablish other electrolyte abnormalities (hypocalcemia, hypokalemia)[37,39]
Sodium, potassium and chloride	Altered	Electrolyte imbalance such hyponatremia, hypochloremia, hypokalemia can be detected in dogs with chronic vomiting. Hypokalemia can be more common as a result of decreased intake and intestinal losses. Electrolyte inversion (hyperkalemia and hyponatremia) instead suggests the presence of concomitant disorders (hypoadrenocorticism, salmonellosis, whipworm infection, or if ascites and third-space effects associated with PLE).[25]
Urea and creatinine	Increased	Pre-renal azotemia (i.e., increased urea and creatinine) will develop if the patient is dehydrated, but an increased urea:creatinine ratio in a fasted animal is suggestive of GI bleeding, with conversion of blood proteins to ammonia by intestinal bacteria, and hence urea formation by the liver. Urinalysis should always be performed in the presence of increased creatinine in order to exclude primary kidney disease.[25]
Folate	Increase or decreased	Folate (vitamin B9) is a water-soluble vitamin present in large amounts in canine and feline diets and is absorbed in the jejunum. In CE serum folate concentrations can increase due to intestinal bacteria production (e.g. consequently to SIBO or EPI or coprophagia)[40] or decrease because of intestinal malabsorption.[4,11,20]

Variable	Alteration	Comment
Cobalamin	Decreased	Cobalamin (vitamin B12) as folate is a water soluble vitamin fully available in diet. Dietary cobalamin is absorbed in the ileum by a specific carrier, the intrinsic factor, produced by the pancreas and stomach. Therefore, cobalamin intestinal metabolism is very dependent on normal exocrine pancreatic and intestinal functions. Decreased serum cobalaminemia is reported in 6% to 73% of dogs with chronic enteropathies[1,11,32] and frequently in dogs with EPI.[4] Another study demonstrated that certain breeds more likely have a decrease cobalamin concentration.[41] Hypocobalaminemia is a negative prognostic factor in CE[1] and patient need oral[42] or parenteral supplementation.[9]
Coagulation profile	Altered	In dogs with PLE an hypercoagulabile state is frequent, leading to formation of thoromboembolic disease. Dogs with PLE have increased fibrinogen, decreased antithrombin III (AT III) and plasminogen activity; and an alteration detectable on thromboelastography (TEG).[43,44]
Urinalysis	Normal or altered	Urine specific gravity (USG) is usually normal in CE, although in some patients it can be decreased. Urinalysis is important in CE diagnostic protocol to exclude some metabolic disorders causing polyuria/polydipsia (PU/PD). Moreover as mentioned above UPC ratio should always be performed in presence of hypoalbuminemia in order to exclude a PLN.

Table 4.a:. Major alterations of parameters available on routine hematology, biochemistry, coagulation profile and urinalysis.

EE, eosinophilic enteritis; SCWT, Soft Coated Wheaten Terriers; PLE, protein-losing enteropathy; GI, gastrointestinal; CE, chronic enteropathies; IBD, inflammatory bowel disease; PCV, packed cell volume; RBC, red blood cell count; UPC, urinary protein:creatinine; PLN, protein-losing nephropathy; TIBC, total iron binding capacity; CRP, C reactive protein; FRE, food responsive enteropathy; cPLI, canine pancreatic lipase immunoreactivity; SIBO, small intestinal bacterial overgrowth; EPI, exocrine pancreas insufficiency; ATIII, antithrombin III; TEG, thromboelastography; USG, Urine specific gravity; PU/PD, causing polyuria/polydipsia.

Chronic GI signs are common in many disorders. Therefore it is important to perform other tests to exclude other differential diagnosis. TLI and PLI are useful to exclude EPI and pancreatitis.[4,20] Finally basal cortisol and ACTH stimulation test are indicated to diagnose Addison disease.[45] Chronic hepatitis and or hepatic insufficiency can be rule out performing blood ammonia, ammonia tolerance test, and fasting and post prandial bile acid.[4]

ABDOMINAL ULTRASOUND

Ultrasonography (US) is an important tool to examine the gastrointestinal tract of small animals with chronic vomiting and diarrhea. Common alterations in dogs with CE are described in Box 4.a. In one study, authors developed an ultrasound score that results to be associated with CIBDAI at time fo diagnosis, but not post treatment.[46] Other authors have evaluated the diagnostic utility of abdominal ultrasound in the diagnosis of GI disorders. That study concluded that abdominal US did not substantially contribute to determination of the diagnosis in the majority of dogs (68%) with chronic diarrhea. In the majority of dogs the diagnosis required endoscopy, or less commonly, celiotomy.[47]

Contrary to the results of this study abdominal ultrasound is always useful in the diagnostic workup of dogs presented for GI signs. It allows the evaluation of concomitant disorders, GI tract appearance (e.g. focal or diffuse lesions, foreign bodies), motility, and appearance of lymphnodes and vessels.

Box 4.a: Common alterations of abdominal ultrasound in dogs with chronic enteropathy.

Diffuse or focal alterations.[48]

Hyperechogenicity of mucosa and hyperechoic spots in mucosa or submucosa and signs of small intestinal lymphangectasia are the principal alterations[48] (see figure).

Wall layering can be maintained or lost. Mucosa, submucosa or muscolaris are mainly affected.[46]

Wall thickening can be present or absent, from mild to severe.[46,48]

Altered intestinal motility, sometime ileus is present, but also increased motility can occur.[49]

Enlarged hypoechic and dishomogenic lymphnodes.

Free peritoneal fluid and peritonel hyperechogenicity.

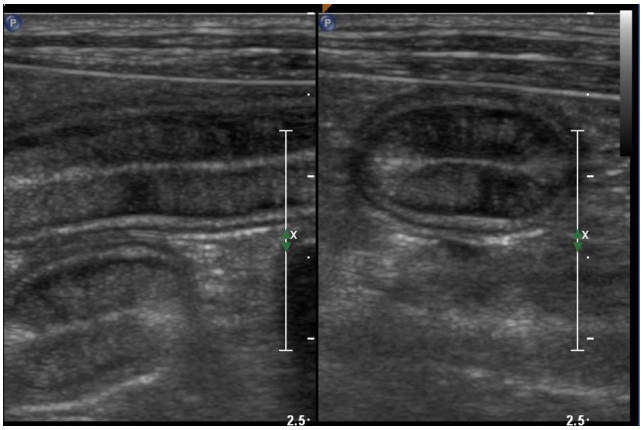

Image of small intestinal lymphangectasia from Ultrasonography archive of Diagnostic Imaging - Department of Veterinary Medical Sciences

ENDOSCOPY

Endoscopy is a medical procedure that permits the clinician to examine the internal structure and to perform diagnostic and therapeutic procedures in a minimally invasive manner (Figure 4.b, 4.c, 4.d and 4.e). Enteroscopy (gastroduodenal or gastroduodenoileumcolonoscopy) is indicated in dogs with chronic or recurrent small or large bowel diarrhea, vomiting, recurrent abdominal pain, weight loss of unknown origin, and signs of gastrointestinal bleeding such as hematemesis, melena, hematochezia, and microcytic, hypochromic anemia. The decision to perform endoscopy should be based on the risk of anesthesia and the likelihood of establishing a definitive diagnosis, prognosis, definitive treatment plan, and positive outcome.[50]

In dogs with suspected CE, endoscopy allows clinicians to visualize the GI tract (esophagus, stomach, duodenum, ileum, colon), the aspect of mucosa, the presence of macroscopic lesions (lymphangectasia, masses, polypus, erosion/ulcers), the presence of foreign bodies, and the collection of biopsies. Endoscopy and histology has not been shown to help in differentiating FRE from ARE or IRE.[1,51]

The majority of dogs do not require immunosuppressant treatment, therefore in most cases, endoscopy may be preceded by dietary and antibiotic trial. Exceptions have to be made in PLE cases and dogs of specific breeds (boxer or French bull dog with signs of colitis) or specific symptoms (emathemesis, melena, regurgitation, and others). Endoscopy or surgery is used to obtain biopsies in poor or non-responders to confirm the presence and type of intestinal inflammation and to rule out diffused intestinal tumors such as lymphoma.[9]

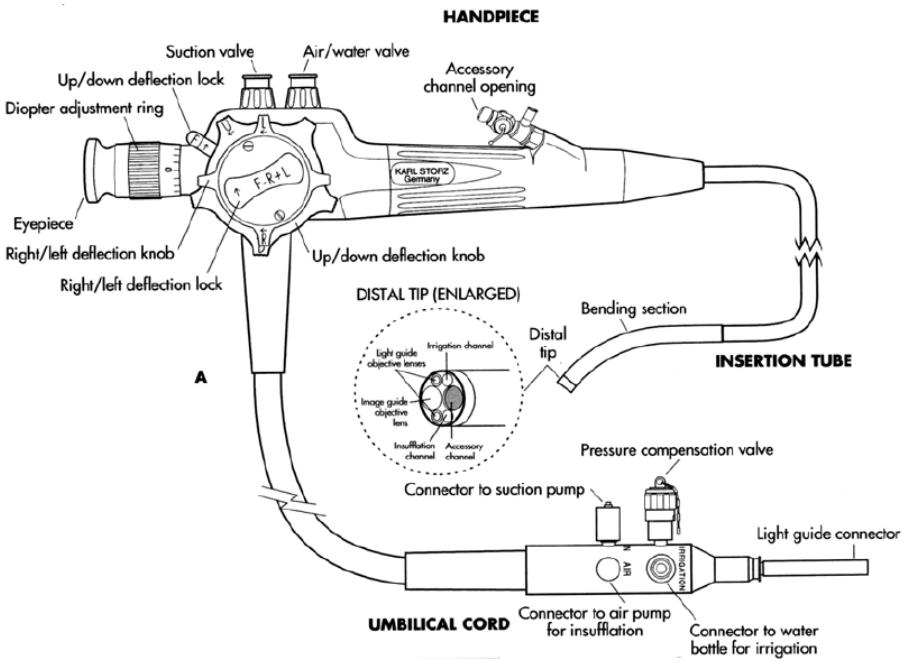

Figure 4.b: Structure of flexible endoscope. From Chamness 2011.[52]

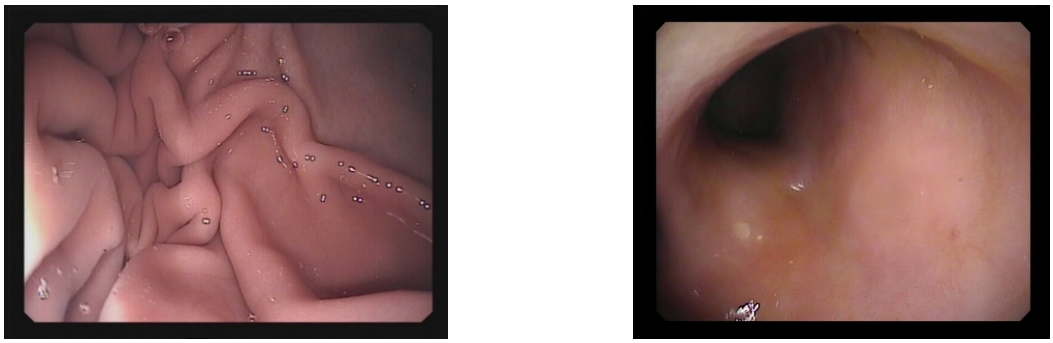

Figure 4. c: Appearance of normal gastric and duodenal mucosa in dogs. From Endoscopic archive of Internal Medicine - Department of Veterinary Medical Sciences

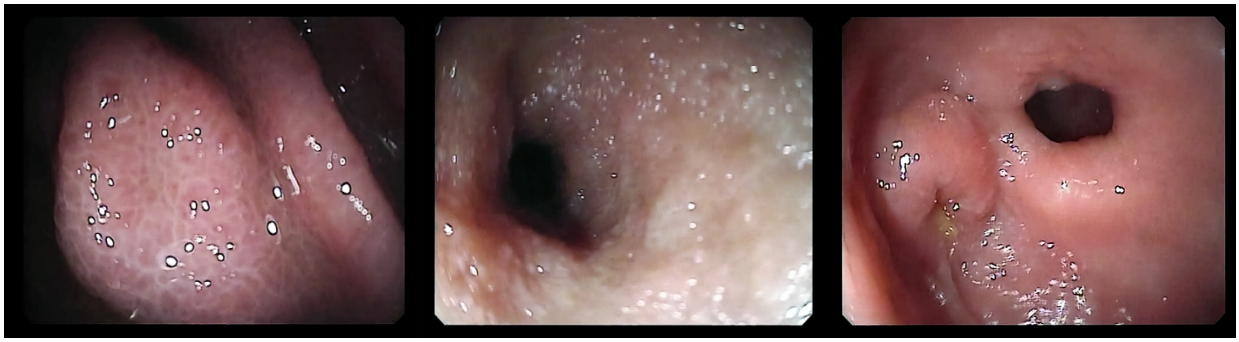

Figure 4.d: Altered gastric mucosa (left image) and altered duodenal mucosa with edema, discoloration, and signs of lymphangectasia (central image), and visualization of ileo-ciecocolic valve with mucosa edema (right image) in dogs. From Endoscopic archive of Internal Medicine - Department of Veterinary Medical Sciences

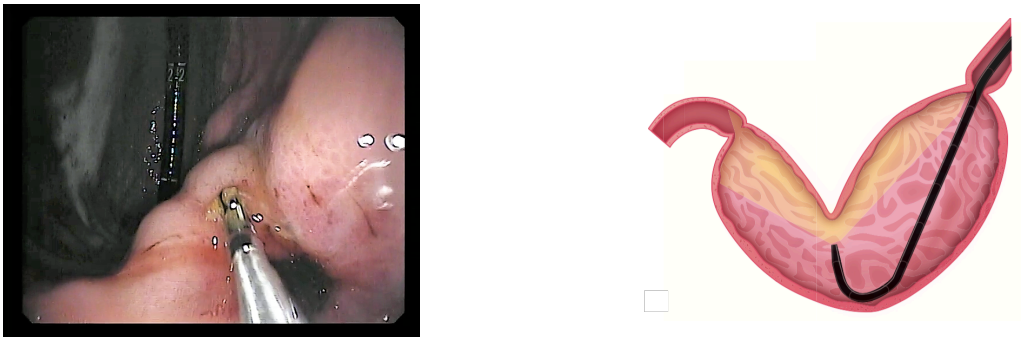

Figure 4.e: Execution of gastric biopsy at level of *incisura angularis* in retroversion (J-maneuver) in a dog. From Endoscopic archive of Internal Medicine - Department of Veterinary Medical Sciences (left image), from Chamness (2013)[50] (right image).

For scientific study purposes and to allow clinicians to have an indication of the severity of endoscopic classification, many endoscopic scores were validated.[53,54] Here the Endoscopic Activity Score (EAS)[54] used in our studies is described.

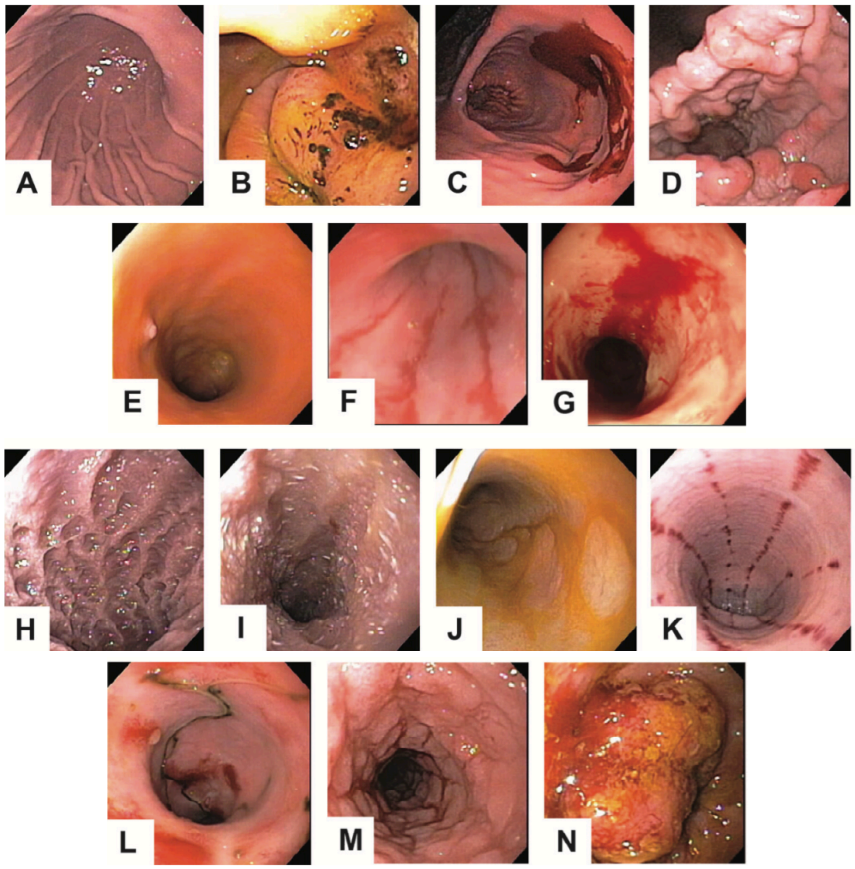

Figure 4.f: Representative still images used in the development phase of the endoscopic study. (A) normal stomach; (B) gastric erosions; (C) gastric friability; (D) gastric granularity; (E) normal duodenum; (F) duodenal erosions; (G) duodenal friability; (H) duodenal granularity; (I) duodenal lymphatic dilatation; (J) normal colon; (K) colonic erosions; (L) colonic friability; (M) colonic granularity; (N) colonic mass. For each characteristic a score in given ranging from 0 to 2. From Slovak et al, 2015.[54]

HISTOLOGY

Histopathologic evaluation of biopsy specimens is required for definitive diagnosis of many intestinal diseases, but has marked limitations, most notably variable quality of tissue specimens obtained endoscopically and poor agreement between histopathologists.[25,53]

The aims of the pathologist are to distinguish normal from diseased tissue, to characterize the nature and severity of tissue changes, and to provide an accurate morphological or etiological diagnosis, thus facilitating formation of a prognosis and appropriate therapy.[53,55] By now, a review of the evidence currently available has not identified a strong association between clinical findings and histopathologic lesions in dogs with IBD, especially when post-treatment changes in disease activity are compared to pre-treatment histopathologic finding.[53]

Forms of small intestinal IBD include lymphocytic-plasmacytic enteritis (LPE), eosinophilic enteritis (EE), granulomatous enteritis, and neutrophilic enteritis. It can be difficult to distinguish severe LPE from lymphoma, especially in cats and when endoscopic biopsy samples are examined.[25]

There is no official convention for the classification of colitis in dogs and cats. Based on histologic patterns, it can be divided into lymphoplasmacytic, eosinophilic, granulomatous (most limited to histiocytic ulcerative colitis (HUC) of Boxer dogs), and ulcerative colitis (may be seen as part of several specific etiologic diseases like HUC of Boxers, or other infectious colitis).[56] Neoplastic disease of the colon is common in dogs, and for this reasons, endoscopic and histopathologic evaluation of dogs presented for large intestine signs is important.

As for clinical and endoscopy scores, also histologic scoring system are available.[53,57] Both the WSAVA histopathologic scoring system[53] and the simplified one[57] integrate cellular infiltration and architectural changes. The simple numerical addition of grades of histopathological change (where normal = 0, mild = 1, moderate = 2, and marked = 3) may provide an overall histological score for the tissue of interest, useful to well-designed studies, well-represented patient populations, and adequate follow- up.[53]

NEW DIAGNOSTIC TOOLS

FECAL CULTURE

Bacterial culture can be a useful technique for detection of specific enteropathogens (e.g. *Salmonella* spp., *Campylobacter jejuni*, *Yersinia* spp.), although primary bacterial gastroenteritis occasionally occurs in dogs. Moreover, positive cultures for enteric pathogens are commonly reported in healthy individuals.[58] Therefore fecal culture, most of the time, is not useful in diagnosis of chronic GI disorders, although sometimes it can be useful, especially in case of acute diarrhea. For acute diarrhea

If possible, collect 2 to 5 g of fresh feces from the rectum.[58] If the dietary history indicates that a raw food diet is being fed, a *Salmonella* culture may be indicated. In some cases, overgrowth of pathogens such as drug-resistant *E. coli* and *Campylobacter* may induce chronic relapsing diarrhea. In such cases, fecal culture may be helpful.

FECAL CYTOLOGY

Fecal cytology is a simple, non-invasive, and cheap test that can be useful in the diagnosis of GI disorders. It can be performed by smear of feces collected for rectal scraping and stained with Diff-Quik (Romanowski stain). Fecal cytology can identify cells such as neutrophils and eosinophils, or pathogens, such as spores of *Clostridium* spp. or *Campylobacter* spp.. Rarely, coccidial merozoites or other protozoal trophozoites are seen.[58]

ALPHA-1PI

α1-Proteinase inhibitor (α1-PI) is a naturally occurring endogenous serum antiproteinase. If lost into the intestinal lumen because of PLE or GI blood loss, it can be found in feces, as it resists bacterial degradation. To improve the diagnostic accuracy of the test, three fresh spontaneously defecated fecal samples should be sampled. Fecal α-1PI is a useful test for the presence of intestinal crypt abscesses and/or moderate to severe intestinal lacteal dilation in dogs. Measure of α1-PI, or fecal to serum α1-PI ratio, allows a prompt diagnosis of PLE in dogs.[25,28,59]

CITRULLINE

Citrulline is an amino acid that is synthesized mostly by intestinal enterocytes and also to some degree by hepatocytes.[3] Citrulline blood concentration level is highly dependent on small bowel enterocytes mass in humans.[60] In dogs, plasma citrulline concentration has been measured[61] and was shown to be significantly decreased in dogs intestinal disease and increased after treatment.[62,63] Based on recent studies, plasma citrulline could be a putative marker of intestinal function in canine IBD.[3,63] Blood concentration of citrulline increase after meal, therefore food should be withheld from dogs for 8 to 12 hours before blood sample collection for measurement of citrulline concentration.[61]

DYSBIOSIS

Dysbiosis is implicated in the pathogenesis of CE in dogs. Assessment of dysbiosis could be important for the diagnosis of CE and several molecular methods are now established for assessing intestinal dysbiosis in dogs and cats, but these approaches are not yet widely available.[64] Methods commonly used for characterization of the intestinal microbiota are: fluorescence in situ hybridization (FISH), quantitative real-time PCR (qPCR), next-generation sequencing (e.g. 454-pyrosequencing, Illumina), metagenomics (shotgun sequencing of genomic DNA). Finally, dysbiosis index is an index that summarizes the abundances of major bacterial taxa in each fecal sample as one single numerical value (a positive number indicates dysbiosis).[64] In the future this index could be useful to recognize dysbiosis and to monitor changes in gut microbiota over time.

FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

The principle of fluorescence in situ hybridization (FISH) is the detection of a target DNA or RNA site by a fluorescently labelled probe molecule. The use of FISH is currently considered to be the most accurate method for quantification of bacterial groups because it allows direct microscopic counting of fluorescence-labeled bacteria. Furthermore, the location of bacteria with regard to the epithelium (ie, intracellular, adherent, or invasive) can be visualized.[64,65]

VITAMIN D

The traditional functions of vitamin D relate to its role in the maintenance of calcium homeostasis and bone metabolism. In humans that vitamin D status is negatively associated with markers of inflammation, and in a number of many chronic diseases. In one study, Vitamin D (serum 25 hydroxyvitamin D concentration) has shown to be inversely associated with markers of systemic inflammation in dogs with CE and with severity of inflammatory changes seen in histopathology samples.[66] Therefore, besides its role in calcium homeostasis, vitamin D could be a new marker of intestinal inflammation.[34,66]

CALPROTECTIN AND S100A12

Calprotectin (S100A8/A9) and S100A12 are validated fecal biochemical markers of intestinal inflammation.[67–69] Canine fecal S100A12 concentrations are increased in dogs with CE and are correlated with the severity of clinical signs, the response to different forms of treatment, endoscopic lesions, and inflammatory changes in dogs with CE.[24,69–71] Finally, the results of another investigation suggested that

fecal S100A12 concentrations have prognostic value in dogs with IBD.[69] Similarly, significantly higher serum calprotectin concentrations have been reported in dogs with idiopathic IBD,[29] and fecal calprotectin correlated with negative outcome and more severe histologic intestinal lesions. [72]

PERINUCLEAR ANTINEUTROPHILIC CYTOPLASMIC ANTIBODIES PERINUCLEAR

Perinuclear antineutrophilic cytoplasmic antibodies (pANCA) have been useful in the diagnosis of human IBD for decades. In dogs, the pANCA assay might be helpful in differentiating dogs with chronic diarrhea caused by FRE or IBD: if the result is positive, a FRE is highly likely, however, if the result is negative, IBD cannot be excluded. [28,73]

pANCA also may be associated with the syndrome of familial PLE in SCWT. However a positive pANCA test result occurs when other inflammatory or immune-mediated diseases are present.[28,74,75]

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5. TREATMENT

In CE loss of tolerance against food antigens and dysbiosis, in genetically predisposed individuals, results to chronic inflammation and consequently malabsorption. Therefore the *core* therapies (dietary modification, antimicrobials, and immunosuppressive treatment) are reviewed here, highlighting the advantages and disadvantages for the most commonly used treatments. Moreover, other products such probiotics and vitamin supplementation commonly used are described. Finally, new therapies are reported because scientific research in treatment of CE is continuously going forward.

THERAPEUTIC TRIALS

A staged approach to therapy is recommended whenever possible, but may not be appropriate in seriously ill patients (e.g., those with PLE) where immediate intervention with combination therapy may be essential. Initial treatment should be always used with anthelmintics agents to eliminate occult parasite infection. Initial treatment with fenbendazole 50mg/kg/day for 3 to 5 days is recommended, along with fecal parasitological exams, as not all parasites are sensitive to this drug (e.g. cestodes) and some are resistance (e.g. *Giardia*).

CORE THERAPIES

DIET

Food allergy and food intolerance fall under the category of an ARF, clinical signs may be identical and their nutritional management is similar.[1–3] FRE can be considered a GI manifestation of ARF.

Once ARF is suspected, the first step is to collect an accurate feeding history from the owners because the elimination diet must not contain any of the ingredients previously given.[4] In most cases, at least 2 commercial diets (one for growth and one for adult maintenance), (dental) treats, table scraps, and human foods used to hide medications have been provided through years and it is not easy to identify all the protein sources to which the dog or cat has come in contact with.[4] Moreover, although animal proteins are mostly suspected as allergens, a complete diet also contains vegetable proteins from grains, fiber sources, or other plants, as well as flavoring factors whose potential in sensitizing the animal should not be underestimated.[9] Moreover, the presence of ingredients not declared in the label are detected in commercial limited-antigens diets.[5,6] Elimination dietary trial can be done with *novel protein* diet (commercial or home-made) or hydrolyzed diet (Boxes 5.a, 5.b, 5.c).[3,7–9] Vegetable proteins can be found in traces in pet food, but are less likely to cause allergic reactions.[4] Therefore a balanced diet with exclusively proteins of vegetable origin could be an alternative elimination diet for dogs with ARF, and is the subject matter of studies of the chapters 5.2 and 5.3.

Apart from *novel* or hydrolyzed protein, a diet for dogs with CE should be high digestible, fat restricted (<15% dry matter), easy to digest fats (e.g. short medium chain triglycerides oil), variable amounts of fiber, and finally ω-3 and ω-6 and prebiotics (FOS, MOS, inulin) can be added.[9]

Fiber content should be carefully evaluated. Soluble fiber (e.g. psyllium) has a role in treatment do dogs with large bowel diarrhea.[10,11] On the contrary in dogs with severe malabsorption fiber content should be low.

Box 5.a: Commercial monoprotein or vegetable diet (extruded and/or wet food)

Advantages	Disadvantages
- Many types of novel proteins, some common (e.g. horse, duck, fish, lamb) some rare (venison, kangaroo, insects) - Easy to use since are balanced and complete, and can be given long term. - Accessible price. - Many options on market, also for growing puppies. - Usually good palatability - Some are added with prebiotics and ω-3 fatty acids.	- Some of the novel protein are so widespread in regular pet food that they can no longer be considered as *novel* proteins (e.g. duck or lamb).[4] - Others protein besides the ones declared can be found.[5,6] - Paucity of studies regarding its use in dogs with CE

Comment: This kind of diet can be considered the *first line* to use in young dogs with early onset of symptoms in which FRE is suspected. Could be considered less suitable for those dogs that have tried many different kind of diets without remission of GI signs. Fat content, in most cases, is not the desirable in diet for dogs with PLE.[12] The diets formulated for *gastrointestinal* problems usually are not suitable options for dogs with CE as contains mainly protein from chicken or turkey sources. Those diets can be an options for acute GI problems or other diseases such acute pancreatitis, although low fat content may be adequate for PLE dogs.[12]

Box5.b: Commercial hydrolyzed diet (extruded and/or wet food)

Advantages	Disadvantages
- Mainly hydrolysate protein from soy, chicken, feather, or fish. - Easy to use, balanced and complete, and can be given long term. - Easy digestible. - Some contain high levels of ω-3 fatty acids.	- Other ingredients can be present[5] - Usually more expensive than other commercial diet. - Palatability is sometimes a problem. - Dogs sensitized to the native protein (e.g. chicken) may still react to the hydrolyzed protein[4]

Comment: Hydrolyzed diet should be considered the *second line*, in those dogs with persistent GI sings that have already tried limited-antigens diets and have failed. Hydrolyzed diets can be the first choice in those dogs with severe GI signs or with PLE, as most of time fat content is adequate.

Box 5.c: Home-made diet

Advantages	Disadvantages
- Quality of ingredients. - Consciousness of the exact ingredients of the diet (from protein to oil and minerals). - The highest palatability and digestibility. - Accessible costs. - Managing disease combinations that do not have a commercially available option (e.g. ARF and renal disease).	- Require time to be prepared. - Costs can be high, depending on body weight of patient and chosen protein source. - Need support by a Nutritionist. - It is mandatory for long period to balance the diet with vitamins and minerals. - Even balanced recipes have not undergone nutrient analysis or food trials.

Comment: As many limited-antigen diets contain other proteins than the ones declared, home-cooked diets should be considered whenever the dog fails to respond to dietary restriction, especially in young dogs in which FRE is suspected. Home-made diet is also the best option when dogs have a decreased appetite or co-morbidity. Balancing a diet with minerals and vitamins is mandatory if the dogs have signs of severe malabsorption (e.g. decrease calcium and vitamin).

BARF (bone and raw foods) diet is adopted by many clients today, but no scientific data support the use of this diet. BARF diet contains a lot of ingredients, and for the most are not complete, so are not suitable for elimination diet in patient with GI problems.[4,13] Moreover it is important to be aware of complication due to bones ingestion, and possible bacterial infection, also with zoonotic potential.[14–16]

ANTIMICROBIALS

The gut microbiota plays an important role in the pathogenesis of IBD, and treatment with specific antibiotics has always been considered a key part in treatment of CE in both humans and dogs (Boxes 5.d, 5.e, and 5.f).[9,17–19] The exact role of the antibiotic remains unclear, although the rational for their use was: decreasing concentrations of bacteria in the gut lumen, altering the composition of intestinal microbiota, and immunomodulatory effect.[17,18,20] Response to antibiotic treatment defines ARE.[19] After cessation of antibiotics, relapses are frequent, but control is typically achieved by reintroducing the antibiotic.[9,20–22] Response both to tylosin and metronidazole seems short-lived. The optimal duration of antimicrobial therapy is still unclear. Usually 4 to 6 weeks is the recommended, although some dogs have relapses and can therefore be treated with low doses for an extended period.[20]

Recent studies demonstrate that antibiotics lead to profound and long lasting changes in intestinal microbiota.[23,24] Therefore in some animals the use of antimicrobial agents is useful (e.g. animals with HUC or EPI), but in others their use may exacerbate dysbiosis.[25] This raises many questions about antibiotics. How useful antibiotics truly are? Are concurrent treatments needed to achieve long-term control? Does long-term, low dose use of antibiotics cause antibiotic resistance?[20] Further studies are needed to understand the role of antibiotic treatment in dogs with CE.

Box 5.d: Tylosin (Macrolide)

Advantages	Disadvantages
- Primary gastrointestinal effect, minimum systemic absorbance. - Response is prompt, some dogs respond within 24 hours, others respond within 3 days. - Antibiotic resistance not demonstrated. - Antimicrobial and immunomodulatory effect.	- Not commercially available. - Not palatable, better capsule formulation rather than tablets.

Dose: There are no official recommendations available for an oral dosage regimen in dogs. Reported treatment doses are: 6-16mg/kg/day, 25mg/kg/day, 15mg/kg BID, 10mg/kg TID, 20mg/kg every 8 to 12h, or 25 to 80 mg/kg from every 8 to 24h.[20,22] In dogs with ARE experiencing relapse a low dose of 5mg/kg/day can be used.[22]

Box 5.e: Metronidazole (Nitroimidazoles)

Advantages	Disadvantages
- Effective against anaerobic bacteria and some protozoal infections. - Systemic effect, good penetration of bones, blood-brain barrier, respiratory tract, and skin. - Metronidazole is rapidly and highly absorbed systemically following oral administration. - Antimicrobial and immunomodulatory effect.	- Resistance to metronidazole is demonstrated but is under-reported. - Neurotoxicity at high doses. (ataxia, lethargy, proprioceptive deficits, nystagmus, and seizure-like signs). Completely reversible in some days. - Not palatable.

Dose: 10-15mg/kg every 12 h (maximum dose 60mg/kg/day).[20,26]

Box 5.f: Others antibiotics

Oxytetracycline: idiopathic ARE responds well to it for its antimicrobial and immunomodulatory effect.

Dose: 10 to 20 mg/kg TID PO and for long-term therapy, low doses can often maintain clinical remission (10 mg/kg SID PO).[21]

Enrofloxacin: HUC of Boxer and French bulldogs is the only CE in which bacterial invasion of the intestinal wall has been clearly documented. HUC has good response to enrofloxacin treatment, although resistance has been described.[20,27,28]

Dose: 7 mg/kg, once a day for 9 weeks.[26]

Rifaximin: is a semisynthetic rifamycin endowed with a wide spectrum of antibacterial activity. It is virtually non-absorbable by oral route, thus granting high efficacy and low incidence of side-effects. This antibiotic is wieldy used in humans, and in one study in dogs it appears to be equally effective to metronidazole.[29]

Dose: 25 mg/kg q12h

IMMUNOSUPPRESSANT

Immunosuppressant drugs are the key treatment in idiopathic IBD and PLE.[9,20] Immunosuppressant drugs include corticosteroids (prednisolone or budesonide), azathioprine, cyclosporine, and chlorambucile (Boxes 5.g, 5.h, 5.i, and 5.j).[20] Other immunosuppressive drugs such as mycophenylate mofetil, methotrexate, and leflunomide have been used to treat immune-mediated or autoimmune

diseases in dogs.[30,31] Due to lack of data and possible side effects on the intestinal mucosa, their use for treatment of IBD in dogs cannot be recommended at this time.
Tacrolimus is related to cyclosporine by its similar mechanism of action and immunosuppression.[31] Tacrolimus is the primary immunosuppressive agent developed for organ transplantation and in humans is used for refractory IBD.[31,32] The clinical use of tacrolimus in veterinary medicine is as a topical therapy for perianal fistulas, keratoconjunctivitis *sicca*, or dermatitis.[31] A recent reports, evaluated its use in a dog with refractory IBD with good results.[32]
Short-term remission rate in IBD dogs ranging from 60 to 80%,[33–36] and long term seems less,[34] but some remain NRE.[20] Therefore evidence now suggests that short-term control of CE seems adequate with a variety of immunosuppressant drugs, but data regarding long term follow up are still missing.[20]

Box 5.g: Glucocorticoids (prednisone, prednisolone and budesonide)

Advantages	Disadvantages
- Prednisolone (prednisone) and budesonide are the most widely used glucocorticoids used in treatment of IBD.[20,30,31,37–39] Dexamethasone can be used in special circumstances.[9] - Systemic impact on both innate and acquired immunity and rapid onset of action. - Side effects are reversible with drugs suspension. Less clinical signs with budesonide. - Low costs for prednisolone (prednisone). Budesonide is more expensive, but still has an accessible price. - May be used in combination with other immunosuppressive agents in refractory IBD.[9,30,31]	- Treating severe canine inflammatory and immune-mediated disease with glucocorticoids often fails in the attempt to achieve disease remission without unacceptable side effects (Figure 5.a). - Adverse effects include iatrogenic hyperadrenocorticism, adrenal gland suppression, gastrointestinal ulceration, insulin resistance and secondary diabetes mellitus, muscle catabolism, delayed wound healing, opportunistic infections, and behavior changes. [31] - P-gp led to resistance of glucocorticoids.[40,41] - Increases the risk of thromboembolic disease. In dogs with PLE an accurate thromboprophylaxis is recommended.[30]

Comment: Glucocorticoids are frequently the first choice of immunosuppressive drugs in treatment of IBD. In middle-large dogs (greater than 25kg) budesonide should be preferred. Optimal duration of treatment with glucocorticoids is not yet defined.[20] The goal of therapy is to achieve clinical remission and slowly taper the dose of glucocorticoids to the lowest dose that controls the inflammatory disease targeted.[31] Often relapses occur and glucocorticoids efficacy can be less. In this case a combination of other immunosuppressive drugs should be chosen.[9,20]

Dose: Prednisone/prednisolone starting dose 1-2 mg/kg every 12h PO.[42]
Budesonide 3mg/m^2 every 24 h[37] (3-7 kg BW 1 mg; 7-15kg BW 2mg; 15-30kg BW 3mg; >30kg BW 5mg)[38]

Figure 5.a: Two dogs with CE showing severe adverse effects to glucocorticoids. One dog (left) shows a severe *calcinosis cutis*, and one dog (right) has a diffuse hair loss.

Box 5.h: Cyclosporine

Advantages	Disadvantages
- Cyclosporine is the only immunosuppressant drug licensed for systemic administration to dogs.[30] - Rapid onset of immunosuppression and the potential for less systemic adverse effects. - Side effects are transient or resolve on discontinuation of the drug.[30] - Concurrent use of ketoconazole increases cyclosporine blood levels, allowing lower doses to be used to reduce costs.[30]	- Side effects are mainly gastrointestinal signs (inappetence and vomiting in a quarter of dogs in the firsts weeks), gingival hyperplasia, and emergence of neoplasia.[20,30,31,42] - Expensive, especially for large dogs.

Comment: efficacy of cyclosporine in treatment of IBD is demonstrated and can be used in dogs with steroid-resistance IBD, with variable effects.[35,43] Effects of cyclosporine in long lasting treatment are known in dogs with atopic dermatitis. Therefore it may be an alternative in patients with relapses or the ones that have to live with immunosuppressant treatment lifelong; but further studies are warranted.[20]

Dose: 3 to 5 mg/kg PO q12 to 24h[35,42]

5.i: Azathioprine	
Advantages	**Disadvantages**
- Low cost. - Used in combination with glucocorticoids, with which it is thought to have a synergistic effect, to allow more rapid dose reduction or tapering of glucocorticoids (the "glucocorticoid sparing" effect).[30]	- Immunosuppressive effects in 7 days to 3 weeks.[30] - The most common adverse effect is myelosuppression that occurs 1 to 2 weeks after therapy, and it is reversible after drug withdrawal.[31] - Regular monitoring of CBC and biochemistry profile is advisable during the first weeks to months of treatment. - Others adverse effects include hepatotoxicity, pancreatitis, and gastrointestinal upset (vomiting and diarrhea).[30,42]

Comment: efficacy of azathioprine is demonstrated in dogs with IBD, and it can be an option in dogs with adverse effects to glucocorticoids to taper the dose. The accessible price is the reason why it is chosen most of the time because adverse effects may be severe.

Dose: 1-2 mg/kg PO q24h[42]

Box 5.j: Chlorambucil	
Advantages	**Disadvantages**
- Accessible price. - Low incidence of adverse effects. - Proven efficacy in PLE dogs.	- Chlorambucil targets B cells and is considered a slow-acting immunosuppressive agent that may require 2 weeks to reach therapeutic efficacy.[31] - Myelosuppression (neutropenia and thrombocytopenia) is considered mild and generally occurs 7 to 14 days after the start of therapy. - A CBC should be performed after 1 and 3 weeks of treatment and repeated every 2-3 months. - Chlorambucil is a chemotherapy, client education is warranted.

Comment: It is used as a *second-line* immunosuppressive therapy in the treatment of IBD that is either severe or poorly responsive to prednisone/prednisolone therapy. One study suggested that a chlorambucil-prednisolone protocol is more efficacious for treatment of PLE, compared to azathioprine-prednisolone combination.[43] There is little evidence for its use in IBD dogs, but it seems to induce a good response.

Dose: 2 to 6 mg/m2 PO q24 to 48h[42]

ADJUNCTIVE THERAPIES

VITAMIN, MINERAL AND ELECTROLYTE SUPPLEMENTATION

CE results in malabsorption that leads to deficiencies in cobalamin, folate, magnesium, and calcium (see chapter 4). Therefore, their integration is always recommended to avoid complications (Box 5.k).

Box 5.k: vitamin, mineral and electrolyte supplementation		
		Route and dose
Cobalamin	-	250-1000mcg once a weeks for 6 week, then 1000mcg once a month SC.[9,44]
	-	1000mcg/day PO.[45]
Folate	-	1 mg of folic acid per day.[21]
	-	1 to 5 mg/dog PO daily.[46]
Magnesium	-	0.75–1 mEq/kg/day IV (0.37–0.5 mmol/kg/day), decreasing to 0.3–0.5 mEq/kg/day IV on subsequent days of magnesium sulphate or magnesium chloride. In emergency situations, a rapid loading dose of 0.15– 0.3 mEq/kg can be given over 15–30 minutes.[47] Attention on renal function and calcium and potassium level.
	-	1 to 2 mEq/kg/day PO of magnesium gluconate, magnesium oxide, magnesium carbonate.[48]
Calcium	-	Parental administration of 10% calcium gluconate (0.5–1.5 mL/kg) is advisable in dogs with acute onset of hypocalcemia related signs.
	-	Vitamin D (calcitriol or 1,25-dihydroxyvitamin D) is recommended in dogs with low ionized calcium to prevent the onset of clinical signs. Dose may be 0.025–0.06 mcg/kg/day, but no definitive dosage is available in literature.[49]
	-	Correction of hypomagnesaemia favorites the correction of hypocalcemia.

THROMBOPROPHYLAXIS

Recent studies have revealed the high prevalence of hypercoagulability in dogs with PLE, which significantly increases the risk of potentially fatal thromboembolic events.[50–59] In dogs with documented or suspected hypercoagulability (panhypoprotidemia, low AT III, thrombocytosis, and glucocorticoids trearment), administration of low doses of aspirin (0.5-1 mg/kg/day) and/or clopidogrel (1-5 mg/kg/day) should be considered in order to prevent thrombosis. However, there is currently no study confirming the beneficial effect of such a therapeutic regimen.[49,60]

PRE- PRO- AND SYMBIOTIC

DEFINITIONS:

Probiotics: are defined as live microorganisms, which when consumed in adequate amounts confer a health benefit on the host.[61,62]

Prebiotics: are defined as selectively fermented ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus also being a benefit to the host organism.[62] These include disaccharides (lactulose, tagatose), oligo- or polysaccharides [fructooligosaccharides (FOS), mannan oligosaccharides (MOS) xylooligosaccharides, polydextrose, galacto oligosaccharides], or long-chain prebiotics like inulin.

Synbiotics: are preparations combining both *probiotics* and *prebiotics*.[62]

PROBIOTICS

Dysbiosis plays a role in the etiopathogenesis of CE (see Chapter 1). This is where the application of pre- or probiotics or their combination (so called synbiotics) has been the focus of much attention in treatment of CE in both humans and animals.[62–64] As probiotics are not usually defined as drugs, they do not have to undergo any process proving their efficacy in applications, diseases, or even target species. Currently, many labeled products containing strains of bacteria are commercially available for treatment of acute and chronic enteropathies, but only few have a proven efficacy (Box 5.l).[62] Moreover, the concentration and viability of the microbiological agent present in available product is sometimes questionable.[65]

Probiotics are shown to have a supportive effect on the microbiota; they may have anti-inflammatory properties and may compete with pathogenic bacteria, reducing the opportunity for those bacteria to adhere to the intestinal mucous membrane and cause further disease (Figure 5.b).[61,62] However, the mechanisms by which probiotics exert their beneficial effects have not been clearly defined.[62]

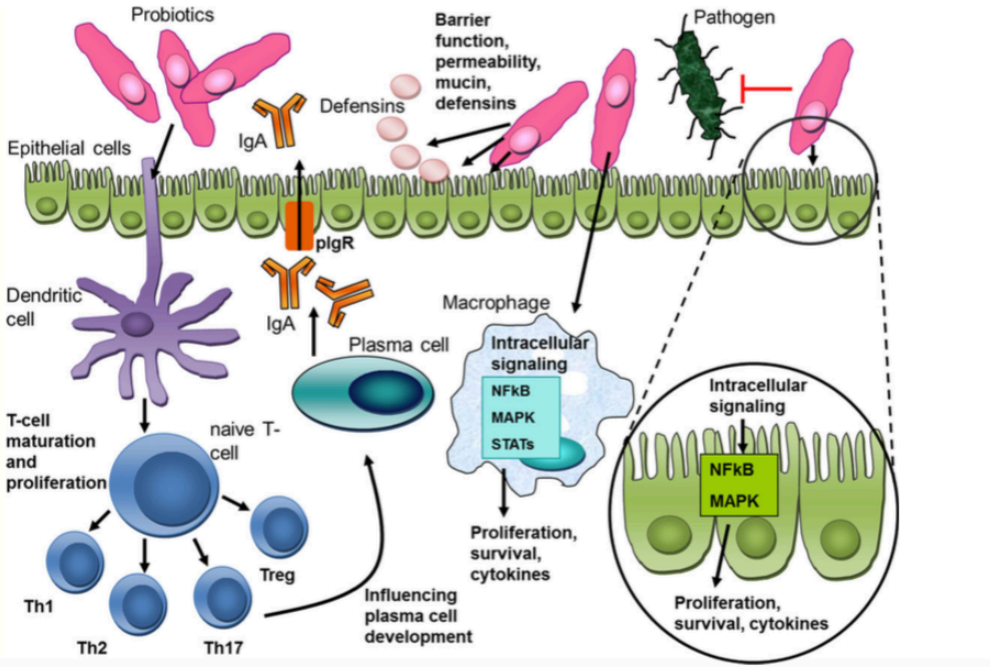

Figure 5.b: Proposed mechanism of action of probiotics. From Schmitz and Suchodolski, 2016[62]

PREBIOTICS

Utilization of prebiotics has several beneficial effects in the canine intestine, including improved composition of intestinal microbiota, reduced concentrations of protein catabolites and enhanced production of SCFA.[66] Furthermore, evidence exists that some positive effects of prebiotics in dogs might be enhanced if these are used in combination with specific probiotic strains, in the form of a synbiotic.[66] Clinical effects of prebiotics have been investigated in humans and animal models but little evidence exists that prebiotics may be helpful in canine diseases.[66]

Box 5.l: Probiotics		
Probiotic	**References**	**Comments**
VSL#3 (Vivomix, Sivoy) Probiotic mixture that includes: *Lactobacilli* (*L. acidophilus, L. plantarum, L. paracasei, L. delbrueckii ssp. bulgar- icus*), *Bifidobacteria* (*B. breve, B. longum, B. infantis*) and *Streptococcus thermophilus*.	**Rossi et al, 2014; Mardini et al, 2014; White et al, 2015.**[67–69]	Used in humans and dogs with IBD and puppies with parvovirus enteritis. Led to clinical and immunological improvement and apparent resolution of dysbiosis.
Enterococcus faecium Two strains approved by European Food Safety Authority *E. faecium* NCIMB 10415 E1705, *E. faecium* NCIMB 10415 E1707)	**Benyacoub et al, 2003; Marcinakova et al, 2006; Simpson et al, 2009; Bybee et al, 2011; Gagné et al, 2013; Schmitz et al, 2014; Schmitz et al, 2015**[70–74]	*E. faecium* has been tested in healthy dogs and puppies. This probiotic was also tested in dogs with giardiasis, acute diarrhea, and FRE with various degrees of efficacy. Finally, a symbiotic contains *E. faecium*, FOS, MOS, and vitamin (B6, B9) was administered to dogs with improvement of clinical signs.
Lactobacilli and bifidobacteria Two approved by European Food Safety Authority *Lactobacillus acidophilus* DSM 13241 25 and *Bifidobacterium* sp. *animalis,*but may others strains have been proven.	**Swanson et al. 2002; Biagi et al, 2007; Chung et al, 2009; Strompfovà et al, 2012; Strompfovà et al, 2014.**[75–79]	Overall data on improving gut health or immunological status in dogs using lactobacilli or bifidobacteria are not compelling.[62]
Saccharomyces boulardii	**McFarland, 2010; Bresciani et al, 2014;**	*S. boulardii* is a non-pathogenic yeast used to treat acute and chronic enteropathies in humans. A recent study concluded by our group indicates that *S. boulardii* can be safely administered to dogs and it might be useful as an adjunctive treatment in CE and PLE (see Chapter 5.1).

NEW THERAPIES

FECAL MICROBIOTA TRANSPLANT (FMT)

Fecal microbiota transplantation (FMT) is the term used to describe the process in which fecal material, or stool, is collected from a tested healthy donor and delivered into a patient either by enema, colonoscopy, or via the upper GI tract (by endoscopy, oral capsules, nasogastric or nasoenteric tube). Nowadays, this definition replaces other names such as stool transplant, fecal bacteriotherapy, and fecal flora reconstitution.[80,81]

In human medicine, FMT is indicated to treat recurrent or resistance *Clostridium difficile* infection.[82] FTP may be indicated for other GI and non-GI disorders, such as Parkinson's disease, chronic fatigue

syndrome, multiple sclerosis, myoclonus dystonia, obesity, insulin resistance, and metabolic syndrome.[80,83] Recently, its usefulness was evaluated in IBD patients with good results.[84]
In dogs and cats, FMT may have the potential to improve health in any disease associated with an alteration or dysbiosis of intestinal microbial ecology such as acute and chronic GI inflammatory disease.[80] However, there is currently very limited scientific data in veterinary patients concerning efficacy, safety, and techniques employed.
In conclusion, based on its remarkable results in humans, the FMT therapy should be further examined and considered as a valid, economic, and potentially highly efficient way of treatment in veterinary medicine.

BILE ACID SEQUESTRANTS

Bile acid (BA) malabsorption is a common yet under-recognized, cause of chronic diarrhea in people; [85] however it has never been investigated in dogs, despite clinical suspicion of its existence.[86] Recent studies suggested that BA malabsorption may be a clinically relevant disorder in dogs with chronic diarrhea.[86,87]
Colestyramine and colestipol are generally effective treatments of gastrointestinal symptoms from BA malabsorption, but their usage is often limited in clinical practice because of their palatability and ability to bind to other medications; other therapies as well as dietary intervention may also have a role.[85]
Colestyramine has been used successfully in both dogs and cats; however, it is not licensed in either of these species. Glucocorticoids have been shown to up-regulate the bile acid transporter, therefore reducing BA diarrhea. Low-fat diets, in humans also reduce secretion of BA. Therefore we can speculate that some dogs with a diagnosis of CE may actually have BA diarrhea, but respond to treatment because corticosteroids and a reduced fat diet are used.[86]
In conclusion, based on human experience and recent data, BA diarrhea should be suspected in dogs with chronic diarrhea, and BA sequestrants may be a therapeutic options. Further studies are needed to explore the clinical significance; clinicians should be aware of the potential for BA malabsorption in cases that are poorly responsive to conventional therapies.[86]

STEM CELLS

Mesenchymal stem cells (MSCs) have shown immunomodulatory and anti-inflammatory effects in animal models of colitis, and promising clinical results have been obtained in humans with Crohn's disease and ulcerative colitis.[88] A recent study in dogs demonstrated that a single IV infusion of allogeneic adipose tissue-derived mesenchymal stem cells was well tolerated and appeared to produce clinical benefits in dogs with severe IBD. [89] Similarly, a recent study of the use of IV allogeneic adipose-derived feline mesenchymal stem cells therapy in spontaneous feline enteropathy showed safety and a positive clinical response.[90]
Both studies had promising results, but further studies are need to assess the efficacy and viability of this treatment in a clinical setting.

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5.1 EFFICACY OF *SACCHAROMYCES BOULARDII* IN DOGS WITH CHRONIC ENTEROPATHIES: DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY

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ABSTRACT

Saccharomyces boulardii is used to treat acute and chronic enteropathies in humans, but to date, no studies have evaluated the use of this yeast in dogs. The current study, a prospective non-randomised, double-blinded placebo-controlled study, evaluated the effects of *S. boulardii* in healthy dogs and dogs with chronic enteropathies (CE).

Four healthy dogs and 20 dogs with CE were included. In healthy dogs, *S. boulardii* was administered for 10 days. Possible short-term adverse effects were recorded, and quantitative stool cultures for yeasts were performed.

In dogs with CE, *S. boulardii* or a placebo was administered in addition to standard treatment protocols. Complete blood work, abdominal ultrasonography, gastroenteroscopy and histology were performed at the time of diagnosis and after 60 days of treatment. In healthy dogs, *S. boulardii* reached a steady state in five days and was completely eliminated on day 4 after administration. No short term side effects were seen. Clinical activity index, stool frequency, stool consistency and body condition score improved significantly in dogs with CE receiving *S. boulardii* versus the placebo.

In conclusion, *S. boulardii* can be safely used in dogs with CE and seems to achieve better control of clinical signs than standard therapy alone.

Key words: *Chronic enteropathy; Dog; Probiotics; Yeast, Saccharomyces boulardii*

INTRODUCTION

Probiotics are live microorganisms that, when consumed in adequate amounts, confer a health benefit to the host (Thomas and Versalovic 2010, Martinez and others 2015, Shmitz and Suchodolski 2016). Numerous studies in many species have shown how a single probiotic strain or combination of strains may modulate gut function and treat several gastrointestinal disorders (Thomas and Versalovic 2010, Rossi and others 2014, Shmitz and Suchodolski 2016). In small animal practice, probiotics are of increasing interest and have a supportive effect on the microbiota. They may have anti-inflammatory properties and may also compete with pathogenic bacteria, reducing the opportunity for bacteria to adhere to the intestinal mucosa and cause further disease (Thomas and Versalovic 2010, Shmitz and Suchodolski 2016). However, the mechanisms by which probiotics exert their beneficial effects have not been clearly defined (Shmitz and Suchodolski 2016).

Saccharomyces boulardii is a non-pathogenic yeast used to treat acute and chronic enteropathies in humans (Mc Farland 2010). Recent studies have investigated its use in treating gastrointestinal (GI) disease in the zootechnical field and in horses (Desrochers and others 2005, Collier and others 2011, Rajput and others 2013, Boyle and others 2013). Although probiotics are used to treat chronic enteropathies (CE) in dogs, the authors could not locate any information in the literature regarding the use of *S. boulardii* in dogs (Thomas and Versalovic 2010).

The current study evaluated the effects of *S. boulardii* in healthy dogs and dogs with CE. The hypothesis was that *S. boulardii* can be administered without any adverse effects and facilitates the control of CE as an addition to standard therapy.

MATERIALS AND METHODS

STUDY DESIGN

First, a prospective clinical trial was performed in healthy dogs to evaluate the viability of *S. boulardii* administration and possible short-term side effects. Then, the therapeutic effects of *S. boulardii* were evaluated in a prospective, non-randomised, double-blinded placebo-controlled study on client owned dogs with newly diagnosed CE. The dogs were administered the probiotic (dose 1×10^9 colony-forming units [CFU]/kg PO q12h) or a placebo, in addition to regular therapies as reported in the literature (Simpson and Jergens 2011). Two galenic formulations were prepared by a private manufacturer, and called Product A and Product B, one of which contained the probiotic and the other contained the placebo without the clinician's or owner's knowledge. Patients were assigned to each group, with the first dog assigned to Group A, and then patients were alternatively assigned to each group (A or B) in a consecutive order. The study design was extrapolated from a previous study (Desrochers and others 2005).

The study was approved by the Scientific Ethics Committee for Experimentation on Animals of Alma Mater Studiorum, University of Bologna (Prot. n.2-IX/9, 2012).

PREPARATION AND QUALITY CONTROL OF *SACCHAROMYCES BOULARDII*

For the study, two galenic formulations were prepared in gelatin-coated capsules. The capsules for the placebo group contained 334 mg maltodextrin. The probiotic therapy formulation (*S. boulardii*) was prepared with capsules containing 10×10^9 CFU of lyophilised *S. boulardii* (523 mg). The capsules for each group were collected from the producer in shaded plastic bins, identified as A or B.

For each lot produced, capsules containing *S. boulardii* were sent from the producer to the Mycology Laboratory of the Department for quality control to confirm the viability of yeast and the titration of *S. boulardii* in the preparation. Briefly, the content of a capsule was dissolved in 100 mL peptone saline diluent (1.0 g peptone, 8.5 g/L sodium chloride; final pH 7.0 ± 0.2 at 25 °C) and incubated at 37 °C for 30 minutes to revitalise the yeast. Ten-fold serial dilutions to 10^{-6} were made from the initial suspension, and 0.1 mL of each dilution was transferred in duplicate and uniformly spread onto the surface of petri dishes containing Sabouraud dextrose agar (BBL Sabouraud dextrose agar, Becton Dickinson and Company, Sparks, MD, USA) with 0.05% chloramphenicol (SAB-CAF). The plates were incubated at 30 °C

for at least 48–72 h. Plates containing fewer than 200 colonies were selected for counting, and the number of CFUs was calculated for each capsule.

Healthy Controls

Client consent was obtained for client-owned dogs of various breeds and ages. The dogs were included in the study if they had a negative faecal parasite examination and a normal physical exam, including an absence of GI signs for at least three weeks before starting the study.

Faecal samples were collected daily for two days (T1, T2) before beginning administration of *S. boulardii*, and were subjected to faecal flotation tests (to assess for intestinal parasites) and to faecal cultures (to exclude the presence of *Saccharomyces* species). Then, *S. boulardii* was administered at a dosage of 1×10^9 CFU/kg PO q12h for 10 days. Dogs were monitored daily through anamnestic investigation, and a physical exam was performed daily. Stool samples were collected every day for the first five days (T1, T2, T3, T4 and T5), and the last day of administration (T10) for faecal cultures to assess the presence of the yeast and to evaluate when *S. boulardii* reached a steady state, defined as 10×10^7 CFU/g of faeces, as described by Mc Farland (2010). Faecal samples were also collected for five days (T11, T12, T13, T14, and T15), as well as on the 10^{th} day after probiotic administration (T20), to confirm the eventual complete elimination of the yeast. Faecal cultures were performed by using an inoculating loop to streak a bit of sample directly onto a plate containing SAB-CAF (direct smear) to evaluate the presence/absence of yeasts and by dissolving 1 g faeces in 9 mL peptone saline diluent, preparing 10-fold serial dilutions to 10^{-6}. Cultures and counts from the dilutions were made as described above for the capsules, to assess CFU/g of faeces. In addition, in all female dogs, a vaginal swab and consecutive yeast culture was performed 10 days after administration (T20) to exclude vaginal colonisation by the yeast.

The API 20C AUX kit (API 20C AUX kit BioMérieux, Marcy-l'Étoile, France) was used for yeast identification according to the manufacturer's instructions. The probiotic tested, on the basis of the assimilation profiles highlighted by the kit, was classified as *S. cerevisiae* because this biochemical test does not provide sufficient evidence to distinguish between strains of this yeast (Rajkowska and Kunicka-Styczyńska 2009). In the Results and Discussion sections, it will be referred as *S. boulardii*.

Dogs with CE

Client consent was obtained for client-owned dogs with CE for inclusion in the study. Inclusion criteria were the presence of chronic GI signs for at least one month before clinical examination, negative faecal parasite examination and/or treatment with fenbendazole (Panacur, MSD Animal Health S.r.l) at 50 mg/kg q24h for five days, and exclusion of other causes of chronic diarrhoea (Jergens and others 1992). Dogs that experienced food-responsive diarrhoea were excluded if they responded to at least two weeks of an exclusion diet (mono-protein commercial diet, hydrolysed diet or restricted home-cooked diet). Dogs with antibiotic-responsive diarrhoea were excluded if clinical signs disappeared after at least two weeks of treatment with antibiotics (tylosin 15 mg/kg PO q12h, or metronidazole 10 mg/kg PO q12h).

All dogs that met the inclusion criteria were subject to an accurate anamnestic investigation, physical examination, complete blood work (complete blood count [CBC], serum biochemistry profile, coagulation profile, and serum folate and cobalamin concentrations), abdominal ultrasound (iU22 ultrasound system, Philips Healthcare, Monza, Italy), gastroenteroscopy (Pentax EG 1840 or Pentax EG 290P, Pentax Italia S.r.L., Milano, Italy) and histology from endoscopic intestinal biopsies. A diagnosis of inflammatory bowel disease (IBD), with or without concomitant hypoproteinaemia (protein-losing enteropathy [PLE]), defined as serum albumin <2 g/dL, normal total protein/albumin ratio, and normal urinary protein:creatinine ratio, was made based on the results of the diagnostic trial.

Dogs were treated with diet (mono-protein commercial diet, hydrolysed diet or restricted home-cooked diet), antibiotics (tylosin 15 mg/kg PO q12h, or metronidazole 10 mg/kg PO q12h), steroids (prednisone 0.5–2 mg/kg PO q24h, in some cases associated with azathioprine 1–2 mg/kg PO q24h or chlorambucil 4–6 mg/m^2 PO q48h) and *S. boulardii* or a placebo, as previously described.

Dogs with CE were followed for 60 days and re-evaluated before (T0) and after histopathologic diagnosis at days 14 (T14), 30 (T30), 45 (T45) and 60 (T60). The validated canine chronic enteropathy clinical

activity index (CCECAI) (Allenspach and others 2007) and body condition score (BCS, 9-point scale) were used to quantify improvements during treatment.
The subgroup consisting of the dogs with PLE were evaluated at T0, T14, T30, T45 and T60 via serum albumin concentration (g/dL).
After 60 days of treatment, complete blood work (CBC, serum biochemistry and coagulation profile), abdominal ultrasound and gastroenteroscopy with histological examination of intestinal biopsies were performed on all dogs. The ultrasonographic appearance of the duodenum and colon was evaluated for the following criteria: wall thickness, wall layering, motility, regional lymphadenopathy, echogenicity changes of mesentery and presence of fluid, by using a scoring system modified by Ripollès and others (2013). The total score of the duodenum and colon was expressed, based on the number of alterations on ultrasound, as normal (no alteration, 0 points), mild (1–2 alterations, 1 point), moderate (3–4 alterations, 2 points) and severe (≥5 alterations, 3 points). Endoscopic images of the duodenum and colon were codified following Slovak and others (2015), and histological findings of the duodenal and colonic biopsies were reported following the World Small Animal Veterinary Association's standardised guidelines (Washabau and others 2010).
The comparison of ultrasonographic, endoscopic and histologic scores of the duodenum and colon, performed before (T0) and after treatment (T60), were used to quantify any improvement during treatment.

Statistical Methods

All statistical analyses were performed using commercially available software (Med Calc 12.2.1.0, MedCalc Software, Ostend, Belgium; Graph prism 5.01, GraphPad Software Inc., La Jolla, CA, USA) with significance designated as $P < 0.05$. The assessment of data for normality was calculated by applying the D'Agostino-Pearson test. Data were expressed as frequency and percentages or median and minimum and maximum.
For dogs with CE, descriptive analysis and comparisons between the group receiving *S. boulardii* and the group receiving a placebo were performed by an independent sample Student's t-test, Mann-Whitney U test or Fisher's exact test, at T0, on signalment (sex, age, body weight, BCS), CCECAI score, laboratory results (CBC, serum total protein, albumin, cholesterol, triglyceride, creatinine, urea, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, folate and cobalamin concentrations), ultrasonographic score, histopathologic score and endoscopic score.
Moreover, CCECAI and BCS were compared between the two groups (*S. boulardii* and placebo) at each time (T14, T30, T45, T60) by an independent sample t-test or Mann-Whitney U test.
Ultrasonographic, histopathologic and endoscopic scores were compared between the *S. boulardii* and placebo groups at T60 by an independent sample t-test or Mann-Whitney U test.
A comparison was performed by the Friedman Test or Wilcoxon test, between T0 and T60, in each of the two groups (*S. boulardii* and placebo) for the following parameters: CCECAI, body weight, BCS, endoscopic score, ultrasound score and histopathologic score.
In patients with PLE, albumin concentration (g/dL) was compared between the two groups (*S. boulardii* and placebo) at each time (T0, T14, T30, T45, T60) by the Mann-Whitney U test, and in each of the two groups (*S. boulardii* and placebo) between T0 and T60 by the Friedman Test.

Results

Quality Control of S. boulardii Capsules

The analysed *S. boulardii* capsules contained the yeast in the concentration declared by the manufacturer.

Healthy Dogs

Four dogs (healthy control: HC1, HC2, HC3, HC4) were included in the healthy group, and consisted of three mixed-breed dogs and one pug. Three of them were spayed females and one was an intact male. Median body weight was 13.5 kg (6.5–21.0 kg) and median age was 66 months (60–84 months). In all

dogs, a faecal parasite examination was negative for the two days before the administration of the probiotic, and faecal cultures were negative for *Saccharomyces* species, even if in three samples colonies of other yeasts were isolated (Table 1). Faecal cultures obtained during the probiotic treatment determined the presence of *Saccharomyces* species concentrations in the stool (Table 1). In particular in all dogs, *Saccharomyces* was present in the faeces from day 1 and reached the steady state (10×10^7 CFU/g of faeces) on day 3 and 4.

The titration of *Saccharomyces* species in faeces decreased rapidly after the withdrawal of treatment, and no colony of *Saccharomyces* species was isolated in any of the dogs four days after treatment (T14), while other yeasts were occasionally found, mainly from direct smears (Table 1).

Yeast cultures from vaginal swabs, performed the 10^{th} day after treatment (T20), were negative for *Saccharomyces* species in all three healthy female dogs. In HC1, a large number of *Malassezia pachydermatis* colonies were isolated.

No short-term adverse effects were reported by the owners. One dog showed signs of mild pain during abdominal palpation the first day of administration of the probiotic. In this case, the clinical sign disappeared after the first day of therapy.

DOGS WITH CE

Twenty dogs with a diagnosis of IBD were initially included in the trial.

The predominant breeds were the German shepherd (5/20; 25%) and Rottweiler (2/20; 10%), and 2/20 (10%) were sexually intact females, 17/20 (85%) were sexually intact males and 1/20 (5%) was a neutered male. The median age of the dogs was 38.5 months (7–108 months), median body weight was 25.9 kg (5.3–64.5 kg) and median BCS was 3/9 points (1–7). Ten dogs (four with PLE) were enrolled in the *S. boulardii* group and 10 (four with PLE) were treated with the placebo. There was no breed prevalence, nor a significant difference in sex, age, body weight and BCS at inclusion between the two groups.

Principal alterations seen on haematological and biochemical exams at inclusion were thrombocytosis (6/20; median 301500/µL [93000-947000], reference interval 160000-500000/µL), hypoalbuminaemia (13/20 had albumin <2.8 g/dL; of these, 8/13 had albumin <2.0 g/dL; median 2.13 g/dL [0.85-3.74], reference interval 2.80-3.70 g/dL), hypocholesterolaemia (8/20; median 150 mg/dL [82-326], reference interval 140-350 mg/dL) and alterations in serum folate (median 14.2 µg/L [2.53-24], reference interval 6.5-11.5 µg/L) and cobalamin concentrations (median 287 ng/L [150-1000], reference interval 250-730 ng/L); 9/16 had increased folate concentrations and decreased cobalamin concentrations and 4/16 had decreased folate concentrations and normal cobalamin.

No significant differences in haematologic and biochemical variables at T0 were detected between the *S. boulardii* and placebo groups, with the exception of serum albumin concentration which was lower in patients with PLE included in the *S. boulardii* group compared to patients with PLE included in the placebo group (median 1.04 g/dL [0.85-1.16] vs 1.61 g/dL [1.22-1.89], P = 0.02).

All dogs received dietary treatment: 9/20 (45%) home-cooked diet, 10/20 (50%) mono-protein pet food diet, and one dog (5%) received both. All CE patients received antibiotics: 18/20 (90%) tylosin, and 2/20 (10%) dogs, in the *S. boulardii* group, received metronidazole. All dogs were treated with prednisolone; 2/20 (10%) and 3/20 (15%) received azathioprine (two dogs in the placebo group) and chlorambucil (two dogs in the *S. boulardii* group and one dog in the placebo group), respectively. There were no detected differences between the groups related to diet and treatment.

The median CCECAI score at inclusion (T0) was 6.5 (5–17) in the *S. boulardii* group and 6.5 (5–16) in the placebo group, with no significant differences between the two groups.

At diagnosis, the median ultrasound score was 1 point (0–3) for the duodenum and 0 points (0-1) for the colon. The median endoscopic score of the duodenum was 4 points (1–5) and of the colon was 0 points (0–4). The median histological score for the duodenum was 9.5 points (3–18) and for the colon was 3.5 points (1–11).

Dogs	Before administration of *S. boulardii*		During *S. boulardii* administration (CFU)						After administration of *S. boulardii* (CFU)					
	T-2	T-1	T1	T2	T3	T4	T5	T10	T11	T12	T13	T14	T15	T20
HC1	–	–	20×10^6	12×10^6	12.2×10^7	56.5×10^7	63×10^6	15.9×10^7	–	–	–	– Rhod)	–	–
HC2	– (Rhod)	– (Rhod)	36×10^4	16×10^6	10×10^3	49.8×10^7	34.4×10^7	30×10^7	1×10^4	1	5 (Rhod)	–	–	–
HC3	– (Cand)	–	16×10^6	20×10^6	35.3×10^7	17.3×10^7	29.3×10^7	11×10^4	2×10^4	1×10^4	–	– (Geo)	–	(Cand; Rhod)
HC4	–	–	12×10^5	90×10^6	15.7×10^7	38×10^5	19.2×10^6	1×10^4	– (Rhod)	–	– (Rhod)	– (Rhod)	–	NP

Table 1 Results of faecal culture in four healthy dogs (HC1, HC2, HC3, HC4) before, during and after *Saccharomyces boulardii* administration (with genera of other yeast colonies isolated via direct smear noted in parentheses).
HC, healthy control; –, Negative; CFU, Colony-forming unit; NP, Not performed; Rhod = Rhodotorula; Cand = Candida glabrata; Geo = Geotrichum

No significant differences were detected between the *S. boulardii* and placebo groups on ultrasound, endoscopic and histologic appearance at T0.
Thirteen dogs reached the end of the study (six in the *S. boulardii* group and seven in the placebo group). Three dogs did not complete the study because of low owner compliance (two after T0 and one after T30), three dogs were euthanised for worsening of clinical conditions (two dogs, one of which had PLE, in the *S. boulardii* group [both after T30] and one dog with PLE in the placebo group after T45). The fifth dog, treated with a placebo and standard therapy, had a good response to treatment but died due to mesenteric torsion (after T30) (Fig 1).

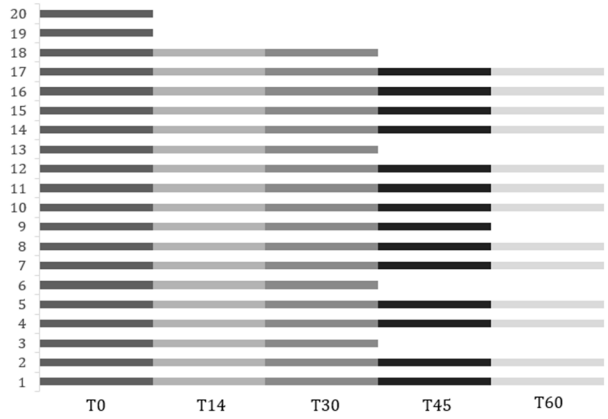

Figure 1. Time line flowchart of patients and dropouts during the study.

All of the data of dogs that left the study were used for statistical analysis when available.
During treatment, both groups showed an improvement in the CCECAI score. In dogs receiving *S. boulardii,* the CCECAI score was significantly decreased ($P < 0.01$) at T14, T30, T45 and T60 compared to T0, and at T45 and T60 compared to T15 and T30.
In dogs receiving the placebo, CCECAI decreased ($P < 0.01$) at T30, T45 and T60 compared to T0. Comparing the CCECAI index of the two groups, dogs receiving *S. boulardii* improved significantly more than dogs receiving a placebo at T45 ($P < 0.05$) and T60 ($P < 0.01$) (Fig 2).

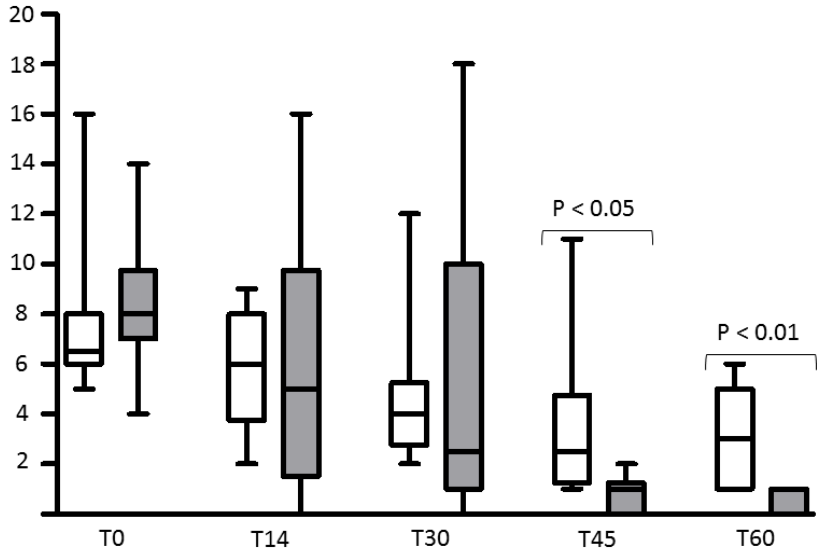

Figure 2. Canine chronic enteropathy clinical activity index (CCECAI) score in dogs receiving Saccharomyces boulardii (gray boxes) or a placebo (white boxes) during treatment. Differences in CCECAI scores were significant between groups at T45 (P < 0.05) and T60 (P < 0.01). Values are represented as minimum and maximum (edges of the bars) median and interquartile range (boxes).

Within CCECAI results, stool frequency was significantly reduced ($P < 0.01$) in the *S. boulardii* group at T30, T45 and T60 compared to T0 and T14; however, no differences were detected in the placebo group. Dogs treated with *S. boulardii* had a significantly lower frequency of defecation at T60 than the placebo ($P < 0.05$).

Stool consistency improved significantly ($P < 0.01$) in the *S. boulardii* and placebo groups at T14, T30, T45 and T60 compared to T0 and returned to normal (point 0) in all dogs at T60 in the *S. boulardii* group, but in only 3/7 dogs in the placebo group, although no significant differences were detected between the groups.

Differences in other CCECAI characteristics (attitude, appetite, vomiting, weight loss, serum albumin, ascites or peripheral oedema, and pruritus) between the two groups were not significant. The BCS increased significantly ($P < 0.01$) only in dogs treated with *S. boulardii* (T45 and T60 versus T0 and T14; and T60 versus T30). Moreover, the BCS at T60 was significantly higher ($P < 0.05$) in the *S. boulardii* group compared to the placebo (Fig 3).

In the six surviving dogs with PLE, serum albumin concentrations at the end of treatment were >2 g/dL in all dogs in the *S. boulardii* group and in 2/3 dogs in the placebo group. Dogs treated with both *S. boulardii* and the placebo showed a significant ($P < 0.01$) increase in albumin concentration (*S. boulardii* group: T0 differed from T30, T45 and T60; T30 differed from T60; and T45 differed from T60; placebo group: T0 differed from T30, T45 and T60; T30 differed from T60; and T45 differed from T60). Comparing albumin concentrations at each experimental time between groups, a statistically significant difference was found only at T0, where dogs in the placebo group had higher albumin concentrations than the *S. boulardii* group ($P < 0.05$). Meanwhile, during treatment (T14, T30, T45 and T60), no difference was found based on albumin concentration.

Data regarding ultrasonography, endoscopy and histology of the duodenum and the colon showed no significant differences before and after treatment, nor between the two groups at the different time points.

None of the dogs with CE showed adverse effects during treatment with *S. boulardii*.

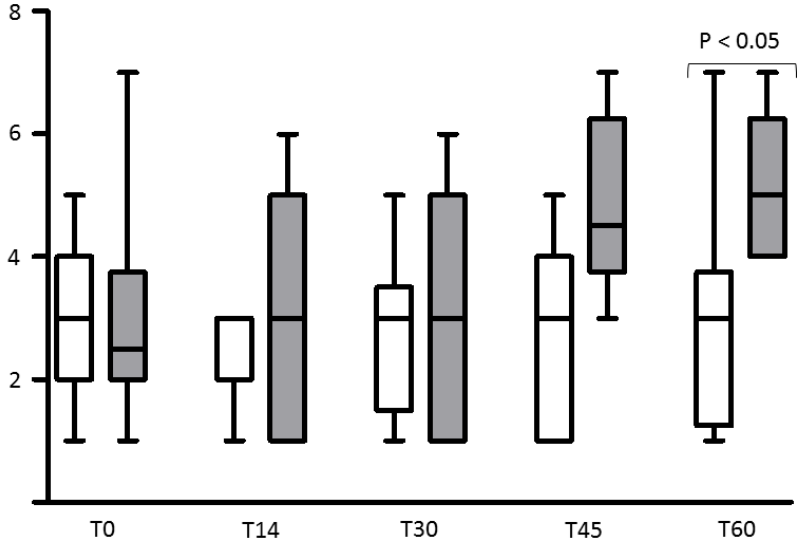

Figure 3. Body condition score (BCS) in dogs receiving Saccharomyces boulardii (gray boxes) or placebo (white boxes) during treatment. Differences in BCS score were significant between the groups at T60 ($P < 0.05$). Values are represented as minimum and maximum (edge of the bars) median and interquartile ranges (boxes).

DISCUSSION

Probiotic treatments in small animal GI diseases are increasing in interest (Schmitz and Suchodolski 2016). Some studies have evaluated the use of a number of strains of bacteria to treat GI disorders (Ménard and others 2004, Sauter and others 2005, Sturm and others 2005, Rossi and others 2014) but, to the authors' knowledge, no study has used this strain of yeast in the treatment of canine CE.

Currently, many labeled products containing strains of bacteria are commercially available for the treatment of acute and CE. One of the main concerns with probiotics is that the concentration and viability of the microbiological agent is sometimes questionable (Weese 2002, Schmitz and Suchodolski 2016). For this reason, part of the current study was dedicated to confirming the presence, viability and concentration of yeast in the probiotic. The authors consider these results interesting because in the first raw material initially provided by a manufacturer to conduct the study, *S. boulardii* was not present in the concentration declared (data not shown). Subsequently, a new product (the one used in the study) was provided and fulfilled the requested criteria.

Doses of *S. boulardii* used in our study were extrapolated from other studies (Weese 2002, Desrochers and others 2005, Mc Farland 2010).

The results from healthy dogs in this study were similar to those demonstrated in human medicine. Administration of *S. boulardii* did not cause any short-term adverse effects. In one study in humans, only 13 cases of 2963 patients analysed reported adverse effects (Mc Farland 2010). The most commonly reported symptoms in people were polydipsia and constipation, and these were not noted in the four healthy dogs included in the present study. In human medicine, a steady state is defined when *S. boulardii* in faeces reaches 10^7 CFU/g of faeces (Mc Farland 2010). In the current study, the same concentration was used to define the steady state.

Before and after administration of *S. boulardii*, the occasional presence of other yeast was not surprising. Despite limited information on the presence of fungal organisms in the GI tract of dogs, the presence of yeast pertaining to the genera *Candida*, *Pichia, Cryptococcus*, *Trichosporon*, *Saccharomyces* and *Rhodotorula* has been previously described (Parle 1957, Suchodolski and others 2008). Nevertheless, it is difficult to determine whether these yeasts are resident fungi or transient as a result of food intake or uptake from environmental sources.

In the healthy dogs, *Saccharomyces* species were not found before administration. The steady state of *S. boulardii* was reached within five days, while in humans it happens three days after the administration of

S. boulardii (Mc Farland 2010). During administration, the concentration of *Saccharomyces* species in faeces of healthy dogs changed between days. One possible explanation for this phenomenon could be related to the different times of faecal production with respect to the administration of the probiotics. Results in humans demonstrate that *Saccharomyces* species have a half-life of six hours (Mc Farland 2010), which could explain the results seen here if we assume a similar half-life in dogs.

It is interesting to note that in all four healthy dogs included in the study, analogous to results in humans (Mc Farland 2010), *Saccharomyces* species in faecal samples disappeared completely four days after withdrawal of the administration.

The results from healthy dogs in this study demonstrate that: 1) *S. boulardii* was absent in faeces of healthy subjects before administration; 2) when administered at a dosage of 1×10^9 CFU/kg PO q12h it caused no short term adverse effects; 3) *S. boulardii* survived in the GI tract and reached steady state in five days; and 4) when the administration was discontinued, it was completely eliminated in four days. Although evaluations were performed in only four dogs and should be interpreted with caution, these results suggest that *S. boulardii* can be safely administrated in dogs.

The population of dogs with CE in this study was similar to what is already reported in veterinary medicine, with German shepherds and Rottweilers being the most commonly represented breeds (Jergens and others 1992). Four dogs died during treatment (three dogs were euthanised because of failure to respond to treatment, and one died due to mesenteric torsion). Sudden death due to thrombosis or abdominal viscera displacement has been reported as a cause of death in dogs with CE (Marks 2013).

In dogs with CE, the results of the current study suggest that *S. boulardii* is effective in improving the control of clinical signs, compared to standard therapy, without short-term adverse effects. All dogs included in the study showed improvements in GI signs, but some significant differences between dogs receiving *S. boulardii* and controls were observed. Similar results have been reported in human studies, in which patients with Crohn's disease treated with *S. boulardii* achieved better control of clinical signs than controls (Plein and Hotz 1993, Guslandi and others 2000). Results in human medicine and animal models demonstrate the anti-inflammatory actions of *S. boulardii* in a large number of diarrhoea disease models. These studies support the notion that the beneficial effects of *S. boulardii* in GI inflammatory conditions are mediated through modulation of host pro-inflammatory responses, not only by whole yeast, but also by secreted factors able to interfere with the host's signaling molecules controlling inflammation at different levels, such as the NF-κB and MAP kinase pathways (Pothoulakis 2009).

At the end of the current study, dogs that received *S. boulardii* had significantly fewer or no clinical signs (stool frequency, stool consistency, CECCAI score) and significantly increased their BCS scores with respect to controls. Moreover, in the absence of clinical and laboratory signs of dehydration, in dogs with PLE, serum albumin concentration seemed to increased more than in placebo dogs. At diagnosis, dogs with PLE that received *S. boulardii* had significantly lower serum albumin concentrations than the control dogs. Otherwise, at T60, all PLE dogs receiving the probiotic had normal albumin concentrations.

In the current study, and similar to what was observed in most previous studies of therapy for canine IBD (Allenspach and others 2007, Simpson and Jergens 2011, Mandigers and others 2010), ultrasonographic, endoscopic and histologic findings did not differ before and after treatment, and consequently, there were no significant differences between the groups. Only in Rossi and others (2014), did the investigators report a difference in histologic examination of canine bowel samples after treatment with strains of probiotics (VLS#3) versus standard treatment.

The current research has some limitations. The first limitation is that a small number of dogs were included in the study, mainly because the owners were reluctant to give consent for repeat endoscopies or, in some cases, the dogs left the study for other reasons. A study with a larger population of healthy dogs and dogs with CE could better characterise the effects of *S. boulardii*, especially regarding dogs with PLE. The second limitation is that the standard therapy was not the same for every patient, even if there were no significant differences in the choice of diet, antibiotics or immunosuppressive drugs between groups.

All patients received follow-up by the same clinician from the first visit to the second endoscopy, and, in most cases, beyond. Therefore, the therapeutic steps were strictly standardised. Future studies should be perfo
rmed in which all dogs receive the same diet, antibiotics and immunosuppressive drugs, but this will require strict owner compliance.

In conclusion, the results of the current study suggest that *S. boulardii* can be safely used in dogs because its administration did not cause short-term adverse effects. In dogs with CE treated with diet, antibiotics and immunosuppressive drugs, *S. boulardii* can be added to achieve better control of clinical signs. Further studies are needed to evaluate the mechanism of action of this probiotic to modify intestinal microflora and invoke an inflammatory response.

CONFLICT OF INTEREST

No third-party funding or support was received in connection with this study, or the writing or publication of the manuscript. The authors declare no conflict of interest, and the study was entirely self-funded.

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5.2 EFFICACY OF AN EXTRUDED VEGETABLE DIET IN DOGS WITH CHRONIC ENTEROPATHIES: CLINICAL PATHOLOGICAL ANALYSIS

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Student thesis, year 2015

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SUMMARY

CE are common disorders in dogs, and food intolerance plays a crucial role in the pathogenesis of this disease.[1,2] Therefore, the aim of this study was to evaluate the efficacy of an extruded vegetable diet in the treatment of dogs with CE. Dogs with a diagnosis of CE was prospectively enrolled in the study (n=20) and divided in 3 phenotypes: FRE (n=7), ARE (n=6) or SRE dogs (n=7).[3] Dogs with SRE included dogs with normal albumin concentration (IBD, n=2) and dogs with albumin concentration less than 2g/dl (PLE, n=5). Dogs were fed a specifically formulated dry food (provided by Effeffe Pet Food S.p.A., Italy) based on the following ingredients: corn, corn gluten meal, potato protein, animal fat, mineral salts, linen seed, fish oil, sunflower oil, beet pulp, dried yeasts, chicory pulp, fructo-oligosaccharides, Yucca schidigera. The chemical composition of the diet (as fed) was the following: water 44 g/kg, crude protein 210 g/kg, crude fat 130 g/kg, crude ashes 49.7 g/kg, starch 345 g/kg. crude fiber 21.5 g/kg. Besides the vegetable diet, dogs included in the trial, with exception of FRE dogs, received antibiotic (in ARE) or antibiotic and immunosuppressive drugs (in SRE).

Dogs included were evaluated at time of diagnosis and after 15, 30 and 60 days (T15, T30, T60). In dogs with SRE, a complete enteroscopy was also performed at time of diagnosis and after 4 months after therapy. To evaluate changes in clinical signs, feces and endoscopy lesions were evaluated with the following available scoring systems: body condition score (9 point, BCS),[4] canine IBD activity index (CIBDAI),[5] fecal score (7 point, Purina, FS), and IBD endoscopic activity score (EAS).[6]

Results show no differences in clinical scores at diagnosis between phenotypes. Otherwise, dogs with SRE had different clinicopathological abnormalities. Dogs with SRE showed a significant increase in platelet count (p=0.041), a significant decrease serum albumin(p=0.018), total protein (p=0.0025), cholesterol (p=0.0011), and total calcium concentration (p=0.0057), and a significant increase in AST serum concentration (p=0.0095).

CIBDAI improved significantly after 15 days in FRE and SRE dogs, and FS improved significantly in all phenotypes (see Figure 5.2a). BCS did not improve during the 60 day trial. No differences between phenotype based on clinical score were noted during treatment, as no dogs had clinical signs after 15 days of diet. EAS did not show any modification of SRE before or after treatment.

Results of this study confirm that severity of clinical signs did not differ between phenotype,[7,8] but clinicopathological alterations are more severe in SRE dogs.[8] Moreover, this study demonstrated that the vegetable diet resulted in a rapid and uniform improvement in clinical signs in all dogs, along with other therapies. The main limitation in this study was the lack of a control group treated with a different diet.

In conclusion, the extruded vegetable diet can be use in dogs with CE in addition to other therapies (antibiotic and/or immunosuppressive drugs) because it results a rapid and uniform improvement in clinical signs.

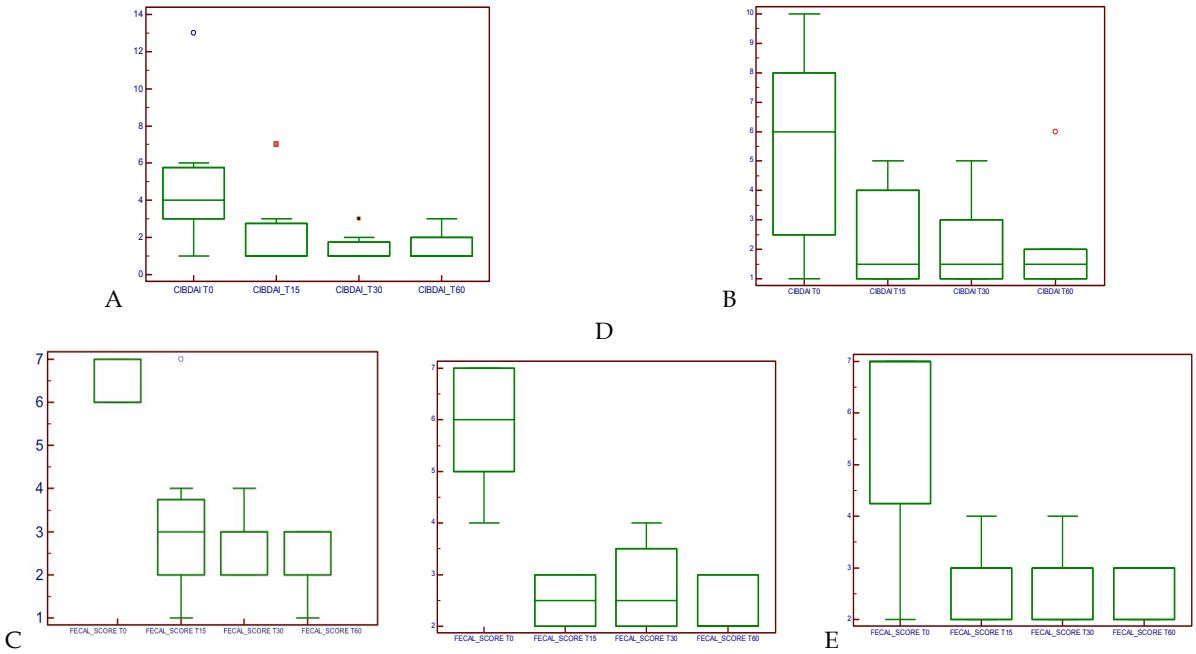

Figura 5.2a. Upper row: Modification of clinical IBD activity index (CIBDAI) in dogs with FRE (A) and SRE (B) after 15, 30, and 60 days of vegetable diet. Lower row: Modification in fecal scores in dogs with FRE (C), ARE (D) and SRE (E) after 15, 30, and 60 days of vegetable diet.

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5.3 EFFECT OF AN EXTRUDED VEGETABLE DIET ON FECAL MICROBIOTA OF DOGS WITH FOOD-RESPONSIVE ENTEROPATHY

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ABSTRACT

Intestinal dysbiosis and adverse food reactions are involved in the pathogenesis of food responsive enteropathy (FRE) in dogs. Various options for an elimination diet are available, and a vegetable dry food is one alternative. Dietary interventions are thought to alter gut microbial communities in healthy individuals and the resolution of dysbiosis is expected in diseased animals concurrent with remission of clinical signs. Therefore, the aim of this study was to evaluate changes in faecal microbiota in dogs with FRE before and after an elimination dietary trial with a vegetable diet. The same vegetable diet trial was performed in healthy control dogs (HC) to evaluate changes in faecal microbiota before and after the trial, and to compare them to FRE dogs.

Dogs with FRE (n=10) and HC (n=14) were fed the vegetable diet for 60 days. Faecal samples were collected before and after the dietary trial. Faecal genomic DNA was extracted and used for Illumina sequencing of 16S rRNA genes. Sequence data were analysed using the QIIME pipeline. The dysbiosis index of the sequence data was calculated using a published mathematical model, and a score >0 was considered as dysbiotic. Statistical significance was set at $p<0.05$.

Significantly lower alpha diversity was observed in dogs with FRE-baseline compared to HC-baseline and FRE-after trial. Distinct microbial communities were observed in dogs with FRE-baseline compared to HC-baseline (ANOSIM p=0.001) and dogs with FRE-after trial (ANOSIM p=0.032). Microbial communities were still different in FRE-after trial compared to HC-baseline (ANOSIM p=0.001). The calculated dysbiosis index was higher in dogs with FRE-baseline compared to HC-baseline (p=0.022), but no significance difference was observed between FRE-after trial and HC-baseline. The faecal microbiota in HC did not show any significant differences before vs. after the vegetable dietary trial.

Results of this study suggest that in FRE dogs, treatment with the vegetable elimination diet led to partial recovery of the faecal microbiota by significantly increasing microbiota richness, which was significantly closer to healthy microbiota after treatment. In contrast, no changes were detected in faecal microbiota of healthy control dogs fed with the same vegetable diet.

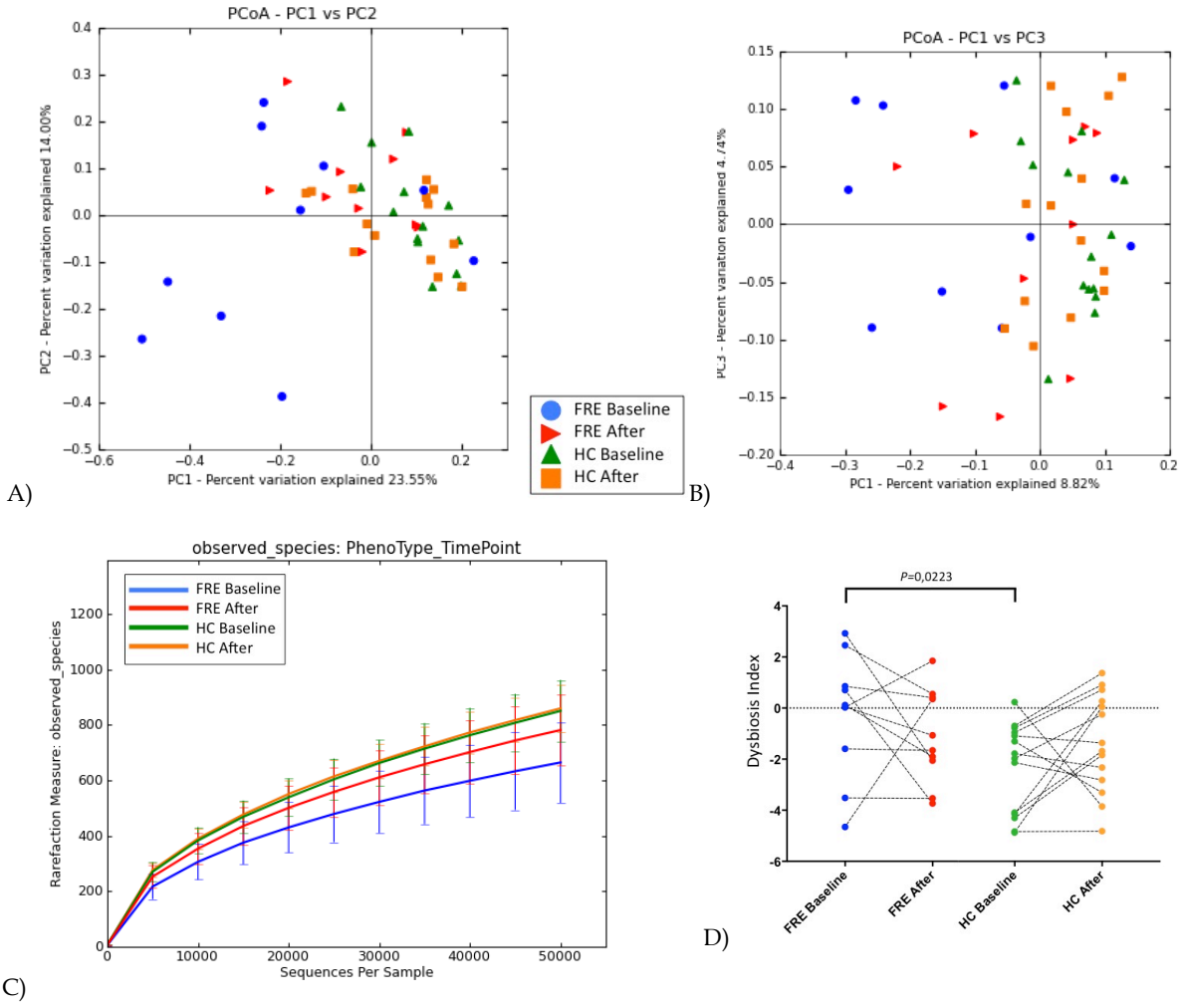

Figure 5.3a: Bacterial diversity measures and microbiota index. Principal finding of sequencing analysis PCoA plot representing beta diversity of microbial communities, based on unweighted (A) and weighted (B) UniFrac distance matrices. Analysis of similarity between groups show significant differences between FRE-baseline and HC-baseline, between FRE-baseline compare to FRE-after, and FRE-after and HC-baseline (C) alpha rarefaction curves of different groups as determined by observed species. Differences were found in dogs with FRE-baseline compared to HC-baseline and FRE-after trial. (D) 16S rRNA sequence dysbiosis index calculated based on abundance of specific bacterial taxa. Paired samples are connected with lines. Significant differences were found between FRE-baseline and HC-baseline.
FRE, food responsive enteropathy: HC, healthy control; Baseline, time first visit; After, after 30 or 60 days of vegetable diet.

5.4 ILEAL AND COLONIC MUCOSAL MICROBIOTA IN DOGS WITH STEROID RESPONSIVE CHRONIC ENTEROPATHIES

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Abstract presented to:2017 ACVIM Forum

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ABSTRACT

Exact etiology for inflammatory chronic enteropathies in dogs remains unknown. Accumulating evidence suggests a pivotal role for intestinal dysbiosis in disease pathogenesis. Many studies have evaluated the alteration of fecal microbiota in canine chronic gastrointestinal (GI) disease, and less research is focused on mucosal microbiota, especially in the ileum and colon. The objectives of the current study were to evaluate ileal and colonic mucosal microbiota in dogs with steroid responsive enteropathy (SRE) before and after 4 months of treatment, and to compare them to control dogs (CD).

A total of 10 dogs diagnosed with SRE were enrolled. Complete GI endoscopy was performed and samples were collected by a cytology brush at diagnosis (SRE-Baseline, n=10) and after 4 month of treatment (SRE-After, n=8). Oral laxative and 2-4 water enemas were performed before endoscopy. A total of 6 CD that were euthanized for reasons unrelated to this study, with no GI disease, were included. Samples from CD were obtained during necropsy within 3 hours of death. Mucosal genomic DNA was extracted and used for Illumina sequencing of 16S rRNA genes. Sequence data were analyzed using the QIIME pipeline. Statistical significance was set at $p < 0.05$.

Clinical signs improved significantly after 4 month of treatment in SRE, but no improvement was seen on endoscopic or histological evaluation. Significant differences in microbial communities between SRE-baseline and CD were observed in the colon (ANOSIM p=0.002), but not in the ileum (ANOSIM p=0.180). In dogs with SRE, both ileal and colonic microbial communities remained similar after 4 month of treatment (ANOSIM p=0.189 and p=0.637, respectively), and were different from CD (ANOSIM p=0.001 and p=0.004, respectively).

Results of this study suggest that the mucosal microbiota in the colon of dogs with SRE is different from that of CD. Although clinical signs improved, colonic mucosal dysbiosis was still present after 4 months of treatment.

6. PROGNOSIS

Prognosis in dogs with CE can be variable. In some dogs it can be favorable after a short period of treatment, such as dogs with FRE or some IBD dogs. On the contrary other dogs, such the ones with IBD or PLE, often fail to respond to treatment and consequently die due to CE or can have several relapses.
In this chapter prognosis and prognostic factors are reviewed.

PROGNOSIS AND SURVIVAL TIME

Prognosis and survival time mostly depends on the phenotype of CE. Usually FRE dogs have a favorable prognosis after a good response to diet. In dogs with ARE no information regarding survival times are available. Most of the studies, instead, evaluate the prognosis and survival time of dogs with IRE-PLE, as this phenotype is the most severe and in all cases prognosis is considered guarded.
In general, a reported prognosis can vary from few days (2 days to some months) to some years (1 to 6-7 years),[1–8] and it depends on many factors that will be reviewed later. For example, some breed such as Shiba dogs, Rottweilers and SCWT seem to have a worse prognosis than other breeds. In Yorkshire Terriers, instead, outcome and survival varied widely; some dogs achieved prolonged survival and remission of clinical signs, whereas others failed to respond.[3,4]

PROGNOSTIC FACTORS

Many prognostic factors have been reported in literature. Prognostic factors can be specific clinical signs or clinical scores, but also clinicopathological, fecal, or histopathological markers.
In Table 6.a available prognostic factors are reviewed.

References	Factor	Findings
MacLachlan et al, 1988; Ohno et al, 2006; Berghoff et al, 2007; Okanishi et al., 2013; Dijkstra et al, 2010; Bota et al, 2016; [2,3,5,7,9,10]	Breed	Specific breeds have a worse prognosis than others, and somehow this could be interpreted as a prognostic factor. Reported breeds with the worse prognosis are: Shiba dog, Rottweiler, SCWT, Yorkshire terrier, Basenji and Norwegian Lundehunds.
Kimmel et al, 2000; Mellanby et al, 2005; Ohno et al, 2006; Goodwin et al, 2011; Dossin and Lavoué, 2011; Simmerson et al, 2014 [4,5,11–14]	Clinical signs	Specific clinical signs are associated with a worse outcome, such as: decreased appetite, severe weight loss (≥ 30%), and vomiting. Ascites, pleural effusion, peripheral edema, thromboembolic diseases and hypocalcemia related symptoms are clinical signs associated with PLE, that usually have a worse prognosis with respect to others phenotypes.
Allenspach et al. 2007; Okanishi et al. 2013; Simmerson et al, 2014; Nakashima et al. 2015; Equilino et al, 2015; Gianella et al, 2017 [1,4,7,15–17]	CCECAI and CIBDAI	High scores of commonly used clinical scoring systems are associated with a negative outcome for some authors but not for others.

References	Factor	Findings
Craven 2004; Ohno et al, 2006; Allenspach et al. 2007; Allenspach, 2013; Equilino et al, 2015; Nakashima et al. 2015 [1,5,15,16,18,19]	Albumin	To many authors hypoalbuminemia is considered a negative prognostic factor in dogs with IBD, but for others it is not. Similarly, an increase in albumin concentration with treatment is considered a good response and it is associated with a favorable outcome.
Allenspach et al, 2007; Batchelor et al, 2007 [15,20]	Cobalamin	Hypocobalaminemia has also been reported to be a negative prognostic factor in dogs with CE or EPI, associated with an increased risk of euthanasia.
Ohno et al, 2006; McCann et al, 2007; Allenspach et al, 2007; Heilmann et al, 2012; Steiner, 2014; Equilino et al, 2015 [1,5,15,21–23]	CRP	CRP seems to correlate with clinical activity score in some studies, but not in others. In one study, CRP failed to correlate with outcome, although for other authors it is useful to assess severity of the disease and monitor the treatment response.
Kathrani et al, 2009 [24]	cPLI	In one study, a proportion of dogs with IBD had high cPLI concentrations and it was correlated with a poor outcome.
Heilmann et al, 2012; Grellet et al, 2013; Heilmann et al, 2014; Heilmann et al, 2014 (1); Equilino et al, 2015; Heilmann et al, 2016 [1,22,25–28]	Calprotectin and S100A12	Higher levels of fecal calprotectin and S100A12 are both associated with negative outcome. Moreover, fecal S100A12 concentration predicts a lack of response to treatment in dogs with CE.
Equilino et al, 2015; Heilmann et al, 2016 [1,29]	α1-PI	α1-PI is an early marker of PLE, but in one study it does not associate with outcome.
Steiner, 2014[23]	N-methylhistamine	N-methylhistamine, is the final metabolite of histamine, and it is an indicator of mast cell inflammation. It can be quantified in serum, urine, or even feces using gas chromatography- mass spectrometry. Fecal N-methylhistamine concentrations have been shown to be significantly higher in dogs with PLE.
Allenspach et al, 2006; Van der Heyden et al, 2011; Allenspach, 2013; Okanishi et al, 2014 [19,30–32]	P-gp	P-glycoprotein (p-gp) is a transmembrane protein functioning as a drug-efflux pump in the intestinal epithelium. High expression of P-gp in T cells of human patients suffering from many neoplastic and autoimmune disorders is associated with poor response to treatment. Similarly in dogs *lamina propria* lymphocyte expression of p-gp is upregulated after prednisolone treatment in dogs with IBD, and low P-gp score before initiation of steroid treatment was significantly associated with a positive response to treatment.
Ohno et al, 2006; Craven et al, 2011; Gianella et al, 2017 [5,17,33]	Response to treatment	Treatment refractory dogs with IBD and granulomatous colitis is associated with a poor outcome. In a recent study no response to treatment one month after diagnosis had a worse prognosis (see chapter 6.2).

Table 6.a: Prognostic factor in chronic enteropathies in dogs.
CE, chronic enteropathies; IBD, inflammatory bowel disease; PLE, protein loosing enteropathies; CCECAI, canine chronic entheropathies anctivity index: CIBDAI canine, IBD activity index; EPI, exocrine pancreas insufficiency; cPLI, canine pancreatic lipase immunoreactivity; α1-PI, alfa 1proteasi inhibitor; P-gp, P- glycoprotein;

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6.1 INFLAMMATORY BOWEL DISEASE IN DOGS: PROGNOSTIC FACTORS FOR THERAPEUTIC RESPONSE

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Abstract presented to: ECVIM-CA Congress, Göteborg, September 2016

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BACKGROUND:

In dogs with idiopathic inflammatory bowel disease (IBD) the response to treatment influences the dog's quality of life, time of survival, economic impact on owners and, sometimes, the decision for euthanasia.

AIM:

The aim of this retrospective single-center study was first to evaluate if response to treatment is able to influence the time of survival, and consequently to evaluate prognostic factors able to influence the response to treatment in dogs with IBD.

MATERIAL AND METHODS

ANIMAL:

Dogs with a diagnosis of idiopathic IBD, treated with immunosuppressive drugs, were enrolled. History about drugs and diet previously employed, clinical signs, laboratory findings, treatment, and follow-up, were recorded. Data from September 2004 to December 2014 were reviewed.

GROUP:

Group 1: were defined as dogs that had responded to treatment, in which immunosuppressive drugs and antibiotic treatment was discontinued without relapses. The group includes dogs alive or dead (for other reasons) that in the moment that were included were receiving any therapies (except diet).
Group 2: were defined as dogs that responded to treatment with immunosuppressive drugs but the disease relapsed after interruption of treatment. This group includes dogs alive or dead (for other reasons) that were constantly on treatment with immunosuppressive drugs or dogs that had relapses treated with immunosuppressive drugs.
Group 3: were defined as dogs that did not respond to diet, antibiotic and immunosuppressive drugs, dog that died spontaneously or were euthanized for correlated cause or dogs alive or dead for other cause with symptoms of IBD not controlled.

STATISTICAL ANALYSIS:

Kaplan–Meier survival curves were obtained for Groups 1, 2 and 3; the survival curves for these groups were compared using the log-rank test. A two stage-analysis was also applied. In the first univariate stage, the variables were screened using x^2 test. In the second stage, factors that screened through $P<0.15$ were evaluated using multivariate logistic regression. The odds ratio (OR) and 95% confidence intervals (95% CI) were calculated from the final model. Receiving operating characteristic (ROC) curve were performed to evaluate cut of value of factor.

RESULTS

One hundred and four dogs met the inclusion criteria.

Dogs of group 1 (n=36) and 2 (n=41) had a median survival longer than dogs of group 3 (n=19), 1215 days (range 210-4380) and 913 days (range 61-3100) versus 210 days (range 30-2005), respectively (Figure 6.1a). At univariate regression analysis, a statistical difference in dogs of group 3 with respect to dogs of group 1 and 2 in following variables, were observed: previous treatment with steroids; weight loss; prevalence of small bowel diarrhea; decreased hematocrit, serum albumin, total protein, creatinine, cholesterol; increased concentration of aspartate amino transferase (AST) and alanine aminotransferase (ALT); and received treatment with other immunosuppressive drugs than steroids at diagnosis. In multivariable model analysis previous treatment with steroids and decreased total proteins were independent variables associated with belonging to group 3. ROC curve of total protein (AUC 0,754) identify a cut off value of ≤ 5.3 g/dl associated with belonging to group 3, with a sensitivity and specificity of 78.9% and 75%, respectively (Figure 6.1b).

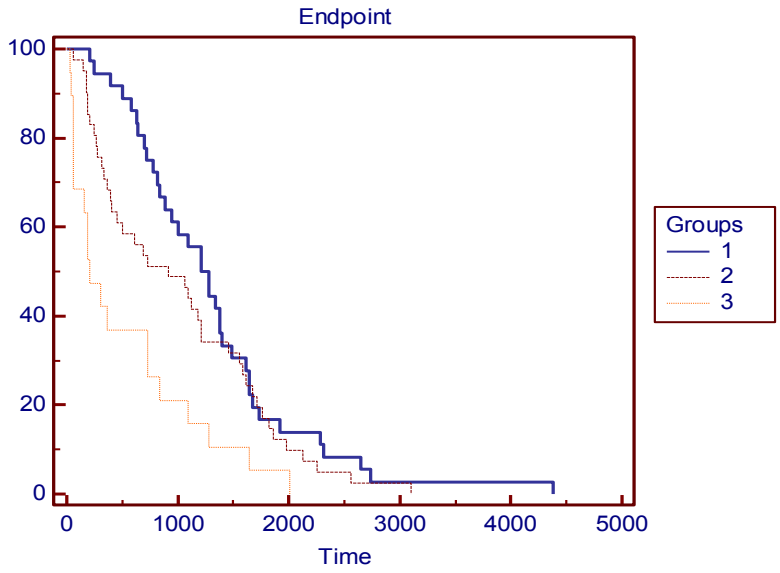

Figure 6.1a: Kaplan–Meier survival curves results of Group 1, 2 and 3. Dogs that responded to therapies, had no relapses and live without drugs (Group 1) had a longer survival time respect to dog that had relapses or are on treatment with immunosuppressive drugs (Group 2), and respect to dogs that did not respond to therapies (Group 3).

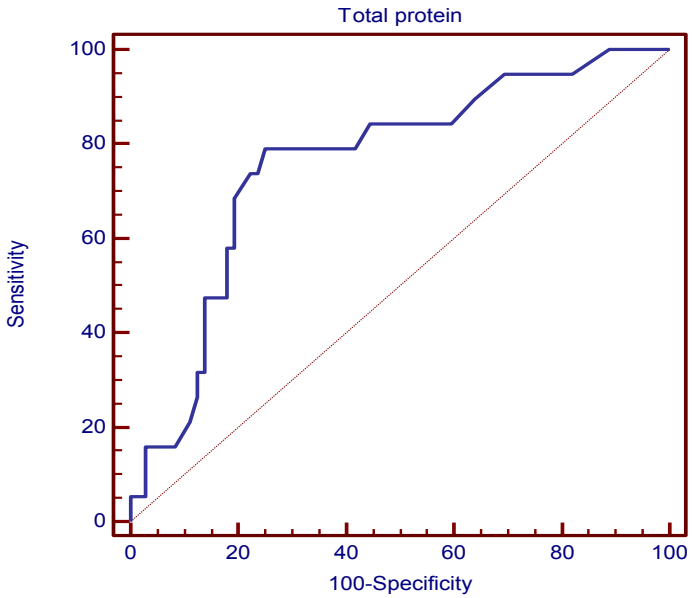

Figure 6.1b: ROC curve analysis of total protein in dogs with IBD (Group 1 and 2 vs Group 3). A cut off value ≤ 5.3 g/dl is indicative of a lack of response to treatment with a sensitivity and specificity of 78.9% and 75%, respectively.

CONCLUSION AND CLINICAL IMPORTANCE

In conclusion, time of survival and response to treatment in IBD dogs was related with previous treatment with steroids, and hypoproteinemia. The first condition could depend on an increased expression of P-glycoprotein (P-gp), a transmembrane drug efflux pump, in lymphocytes infiltrating the intestinal *lamina propria*.[1] High expression of P-gp has been associated with a less response to steroid treatment.[1] The second negative prognostic factor for therapeutic response (hypoproteinemia) is already known to be related with a negative outcome.[2] In summary, our study demonstrates that, over 35 parameters exanimated, only 2 (previous treatment with steroids, along with decreased serum total proteins at beginning of treatment) are negative prognostic factors in dogs with IBD and are associated with a lack of response to treatment, and therefore a shorter survival time.

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6.2 CLINICOPATHOLOGIC AND PROGNOSTIC FACTORS IN SHORT- AND LONG-TERM SURVIVING DOGS WITH PROTEIN-LOSING ENTEROPATHY

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ABSTRACT

The aim of the present study was to investigate the differences in the characteristics of short- and long-term surviving dogs with protein-losing enteropathy (PLE) and to identify factors that predict its outcome. We retrospectively reviewed the medical records of 59 client-owned dogs with PLE diagnosed at three different hospitals between January 2009 and November 2013. The dogs were classified as either short-term (≤ 6 months; STs) or long-term (> 6 months; LTs) survivors. Clinical and clinicopathological variables were investigated between the groups and receiver operating characteristic (ROC) curve analysis was performed. Nineteen dogs were classified as STs and 40 as LTs. Body weight and blood urea nitrogen concentrations were significantly higher in the STs at diagnosis ($P < 0.05$). At 1 month after initiation of immunosuppressive therapy (data-driven cut-off, T1), chronic canine enteropathy clinical activity index (CCECAI) scores were higher ($P < 0.01$) and albumin, serum total protein and total cholesterol concentrations were lower ($P < 0.01$) in the STs. ROC curve analysis showed that CCECAI > 5 evaluated at T1 was the best predictor of poor outcome. Although the severity of clinical signs and the majority of clinicopathological findings at diagnosis did not influence the outcome, survival time was shorter in the dogs with high CCECAI scores at T1 and that did not respond to therapy.

Keywords: *canine, inflammatory bowel disease, chronic enteropathy, risk factors, outcome*

INTRODUCTION

Protein-losing enteropathy (PLE) in dogs results from severe small intestinal disease that allows leakage of protein into the intestinal lumen (Dossin and Lavoue, 2011). While panhypoproteinemia associated with loss of albumin and globulin is the most common clinicopathological abnormality, isolated albumin loss can also be observed (Willard et al., 2000; Allenspach et al., 2007). The major causes of PLE in dogs are intestinal lymphangiectasia, inflammatory bowel disease, and lymphoma (Craven et al., 2004; Dandrieux et al., 2013; Nakashima et al., 2015). Because PLE is associated with decreased serum albumin and increased loss of α1-PI into the gastrointestinal tract, measurement of serum albumin and fecal α1-proteinase inhibitor (α1-PI) should be included in the diagnostic workup (Murphy, 2003; Willard, 2013). But since the α1-PI test is not readily available, PLE is usually diagnosed after excluding other conditions associated with hypoalbuminemia and intestinal histopathology (Dossin and Lavoue, 2011; Willard, 2013). As compared with chronic enteropathy (CE) with normal albumin (Craven et al., 2004; Allenspach et al., 2007; Simpson and Jergens, 2011), the prognosis for PLE is usually considered guarded (Allenspach et al., 2007; Dossin and Lavoue, 2011), and the response to therapy is variable (Simmerson et al., 2014).

Moreover, information on factors that predict outcome of PLE at diagnosis is limited and long-term follow-up data are lacking. Negative prognostic indicators include medium size (11 to 20 kg), high canine IBD activity index (CIBDAI) score, a history of vomiting, monocytosis, mildly increased C-reactive protein, normal serum calprotectin and S100A12 concentrations, clonal rearrangement of lymphocyte antigen receptor genes, and intestinal villous blunting (Simmerson et al., 2014, Equilino et al., 2015; Nakashima et al., 2015). Information on the impact of serum albumin and blood urea nitrogen concentrations on outcome or survival time is controversial. One study found the survival time to be significantly influenced by low blood urea nitrogen concentration and severity of hypoalbuminemia (Simmerson et al., 2014), two others reported that elevated blood urea nitrogen concentration and hypoalbuminemia, but not its severity, were negatively correlated with outcome (Owens et al., 2011; Nakashima et al., 2015), and another found that outcome or survival time were not significantly influenced by the initial serum albumin concentration (Equilino et al., 2015). Finally, while many dogs with PLE secondary to CE die shortly after initiation of treatment, there are some that achieve prolonged survival.

The aim of this study was to retrospectively evaluate the differences in clinical and clinicopathological findings between short- and long-term surviving dogs with PLE secondary to CE at diagnosis and after treatment, and to identify potential risk factors for poor outcome.

ANIMALS, MATERIAL AND METHODS

HISTORY AND LABORATORY FINDINGS

We retrospectively reviewed the medical records of dogs with PLE secondary to CE diagnosed at three different hospitals between January 2009 and November 2013. Inclusion criteria were complete history and physical examination findings, chronic gastrointestinal signs lasting for more than 3 weeks, hypoalbuminemia (< 2 g/dL) of gastrointestinal origin with or without hypoglobulinemia, and histopathological evidence of gastrointestinal inflammation on biopsies collected by endoscopy or laparotomy. Histologic examination was performed in all dogs according to the histopathological standards of the World Small Animal Veterinary Association (WSAVA) Gastrointestinal Standardization Group. All biopsies were retrospectively reviewed by a pathologist blinded to the diagnosis. Chronic canine enteropathy clinical activity index (CCECAI) scores (Allenspach et al., 2007), complete blood count, serum biochemistry and coagulation profiles, pancreas specific lipase levels, serum folate and cobalamin concentrations were gleaned from the medical records. The reference ranges of the hospital laboratories were substantially similar. During review of the medical records (February 2015), follow-up information was obtained by telephone from the owners or referring veterinarians.

CLASSIFICATION AND THERAPY

Dogs were classified as either short-term (STs) or long-term (LTs) survivors if they had died within or were still alive at 6 months after diagnosis, respectively. Additionally, the medical records were searched for information about the categorization of CE as food-, antibiotic-, or immunosuppressive-responsive. Dogs that showed complete remission of clinical signs while on elimination diet (hydrolysed or restricted antigen diets) were categorized as having food-responsive CE. Dogs that showed complete remission of clinical signs while on tylosin (15 mg/kg, PO, q 12 h) or metronidazole (10 mg/kg, PO, q 12 h) were categorized as having antibiotic-responsive CE. Dogs that responded to oral prednisone (1 mg/kg, twice a day for 2-3 weeks before considering dose reduction), oral azathioprine (1 or 2 mg/kg, once a day), oral chlorambucil (4-6 mg/m2, once a day for at least 2 weeks before considering dose reduction), or oral cyclosporine (5 mg/kg, once a day) were categorized as having immunosuppressive-responsive CE. Dogs were classified as immunosuppressive-unresponsive if they showed poor or no clinical response to immunosuppressives (partial disappearance or persistence of clinical signs).

Since the medical records also reported the results of repeated exams at follow-up visits , we set T1 (1 month after initiation of immunosuppressives) as the time point at which the clinical and clinicopathological information was complete for the majority of the dogs.

STATISTICAL ANALYSIS

Statistical analysis was performed with a commercially available statistical data analysis program (MedCalc®). Assessment of data for normality was calculated using the D'Agostino-Pearson test. Continuous variables were expressed as mean (± sd), median (minimum and maximum), percentages or both. Categorical variables were expressed as normal/negative (0) or abnormal/positive (1). Fisher's exact test was used to compare between the STs and the LTs the variables: sex, complaints/clinical signs (small bowel diarrhea, mixed diarrhea, decreased appetite, increased appetite, vomiting, peripheral edema, ascites, pleural effusion, pruritus, polyuria and polydipsia, lethargy, and muscular twitching/convulsions), results of the SNAP cPL® test, coagulation profile at T0, and treatments with different types of immunosuppressives.

Student's t-test was used to compare between the STs and the LTs the variables: age, body weight, CCECAI scores, serum albumin, folate concentrations, and lipase activity at T0 and the CCECAI scores, serum albumin and globulin concentrations at T1. The Mann-Whitney test was used to compare between the STs and the LTs the variables: number of monocytes and platelets, globulin, serum total protein, total cholesterol, blood urea nitrogen, magnesium, cobalamin and fibrinogen concentrations at T0 and the serum total protein, magnesium and total cholesterol concentration at T1. Values of $P < 0.05$ were considered significant. A receiver operating characteristic (ROC) curve was used to select the optimum cut-off value of the variables at T1 to discriminate the STs from the LTs.

RESULTS

HISTORY, PHYSICAL EXAMINATION, AND CCECAI SCORES

We reviewed the medical records of 59 dogs with PLE secondary to CE diagnosed between January 1, 2009 and November 30, 2013. Of these 59 dogs, 19 were classified as STs and 40 as LTs. Among the STs were dogs from 9 different breeds and 2 mixed-breed dogs. Fourteen were males and 5 females. The age range was from 9 months to 13.4 years (mean, 5.9 ± 3.3), and the weight range was from 14 to 40 kg (mean, 23.6 ± 7.3). Table 1 reports the presenting complaints/clinical signs. The median duration of clinical signs prior to diagnosis was 2 months (range 1-36). The median survival time was 90 days (range 31 to 180). Among the LTs were dogs from 20 different breeds and 9 mixed-breed dogs. Twenty-two dogs were male and 18 female. The age range was from 1 to 11.6 years (mean, 6.5 ± 2.5), and the weight range was from 1.9 to 45 kg (mean 17.4 ± 12.4). Table 1 reports the presenting complaints/clinical signs.

Variables	STs (n/t)	LTs (n/t)
Small bowel diarrhea	19/19	39/40
Mixed diarrhea	5/19	14/40
Decreased appetite	13/19	22/40
Increased appetite	1/19	3/40
Vomiting	9/19	24/40
Peripheral edema	1/19	5/40
Ascites	6/19	20/40
Pleural effusion	-	3/40
Pruritus	1/19	4/40
Polyuria and polydipsia	4/19	5/40
Lethargy	2/19	8/40
Muscular twitching/convulsion	1/19	4/40

Table 1. List of presenting complaints/clinical signs at diagnosis (T0) in short-term (ST) and long-term (LT) survivors.
n= number of dogs showing the complaint/clinical sign; t= total number of dogs

The median duration of clinical signs prior to diagnosis was 2 months (range 1-36). The median survival time was 880 days (range 210 to 1,787). No statistically significant differences in sex and age between the two groups were found at T0; body weight was significantly higher in the STs ($P < 0.05$). There was no difference in presenting complaints/clinical signs between the STs and the LTs.
CCECAI scores were available for all dogs at T0, and for all dogs except 1 at T1. No significant differences in the CCECAI scores between the groups were found at T0; at T1 the CCECAI score was significantly higher in the STs (Figure 1).

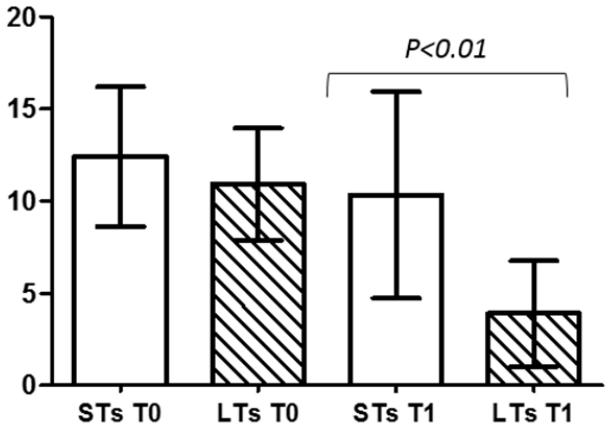

Figure 1: Comparison of canine chronic enteropathy activity scoring index (CCECAI) between short-term (ST) and long-term (LT) survivors at T0 and T1.

CLINICOPATHOLOGICAL FINDINGS

Tables 2 and 3 present the clinicopathological findings and the number of dogs that had undergone testing, respectively. At T0, no statistically significant differences between the two groups were found for: number of monocytes and platelets, serum albumin, globulin, total protein, total cholesterol, magnesium, cobalamin, folate and fibrinogen concentrations, lipase activity, results of the SNAP cPL® test, and coagulation profile. Blood urea nitrogen concentrations were significantly higher in the STs ($P < 0.05$). At T1, albumin, serum total protein and total cholesterol concentrations were significantly lower in the STs ($P < 0.01$).

Variables	STs		LTs		Reference values
	Positive or abnormal	Mean (±SD)	Positive or abnormal	Mean (±SD)	
Albumin	-	1.49 (±0.42)	-	1.45 (±0.31)	2.80-3.70 g/dL
Globulin	-	2.08 (±0.87)	-	1.88 (±0.61)	2.80-4.20 g/dL
Total Protein	-	3.57 (±0.97)	-	3.32 (±0.75)	5.60-7.90 g/dL
Total Cholesterol	-	118 (±54)	-	122 (±48)	140-350 mg/dL
Magnesium	-	1.67 (±0.86)	-	1.88 (±1.90)	1.60-3.20 mg/dL
Blood Urea Nitrogen	-	37.62 (±18.65)	-	28.72 (±17.32)	18-55 mg/dL
Cobalamin	-	219 (±128)	-	229 (±107)	250-730 ng/L
Folate	-	9.95 (±5.80)	-	10.42 (±5.90)	7-17 µg/L
Fibrinogen	-	450 (±146)	-	555 (±225)	150-450 mg/dL
Lipase	-	191 (±123)	-	302 (±205)	70-700 U/L
SNAP cPL®	2	-	3	-	-
Platelets	-	463,684 (±218,761)	-	426,056 (±252,977)	150,000-500,000 cells/µL
N of monocytes	-	686 (±482)	-	634 (±396)	100-1400 cells/µL
Coagulation profile	9	-	10	-	-

Table 2. Summary of laboratory results at diagnosis (T0) in short-term (ST) and long-term (LT) survivors.

Variable	T0		T1	
	STs (n/t)	LTs (n/t)	STs (n/t)	LTs (n/t)
Albumin	19/19	40/40	18/19	40/40
Globulin	19/19	40/40	18/19	39/40
Total Protein	19/19	40/40	18/19	39/40
Total Cholesterol	19/19	38/40	13/19	31/40
Magnesium	9/19	26/40	6/19	18/40
Blood Urea Nitrogen	19/19	37/40	-	-
Cobalamin	13/19	30/40	-	-
Folate	13/19	32/40	-	-
Fibrinogen	5/19	16/40	-	-
Lipase	10/19	27/40	-	-
SNAP cPL®	5/19	17/40	-	-
Number of platelets	19/19	38/40	-	-
Number of monocytes	18/19	32/40	-	-
Coagulation profile	6/19	13/40	-	-

Table 3. Summary of clinicopathological variables tested at diagnosis (T0) and 1 month after initiation of immunosuppressive therapy (T1) in short- term (ST) and long-term (LT) survivors.
n=number of dogs in which the variable was measured; t=total number of dogs

GASTROINTESTINAL HISTOPATHOLOGY RESULTS

Gastroduodenoscopy was performed in 58 dogs. Additional ileoscopy and colonoscopy were performed in 14 and 28 dogs, respectively. Laparotomy was performed in 1 dog. Tissue quality was classified as adequate in all cases. Moderate to marked histopathologic abnormalities in the small intestine were found in all dogs. Lymphocytic-plasmacytic inflammation (50 dogs) and lymphangiectasia (28 dogs) were the most common abnormalities. Moderate to severe lymphocytic-plasmacytic colonic inflammation was found in 24 dogs.

TREATMENT AND OUTCOME

No significant differences in the treatments with different types of immunosuppressive therapies were found between the two groups. Based on their response, all STs were categorized as immunosuppressive-unresponsive. Among the LTs, 32 dogs were categorized as immunosuppressive, 1 and 1 each as food- and antibiotic-responsive CE, respectively; 6 dogs were categorized as immunosuppressive-unresponsive.

Follow-up information was available for all dogs. Thirty-three dogs (55.9%; 31 with immunosuppressive-responsive CE; 1 with food-responsive CE; 1 with antibiotic-responsive CE) were alive at the time of medical record review (73 months), and 26 (44.1%; 19/19 STs and 7/40 LTs with immunosuppressive-responsive CE) had died because of PLE-related complications. The main cause of death was deterioration of clinical conditions presumably due to malabsorption. A cut-off CCECAI score of > 5 at T1 was found to be the best predictor for poor outcome (Figure 2).

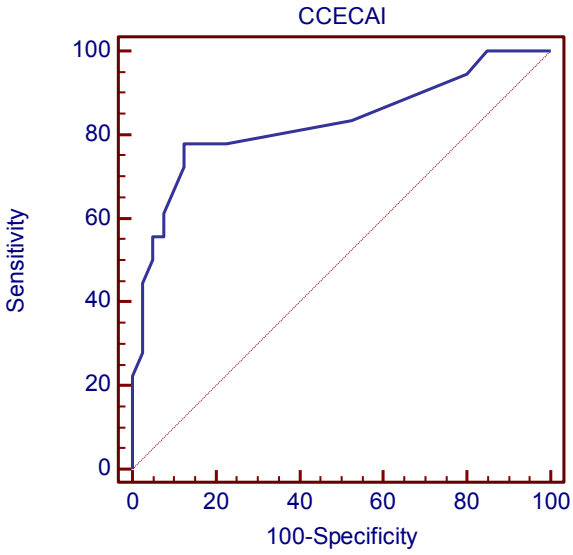

Figure 2: The receiver operating characteristic (ROC) curve used to select the optimum cut-off value of the variable CCECAI associated with survival to discriminate between short-term (ST) and long-term (LT) survivors.

DISCUSSION

With this retrospective multicenter study we compared the clinical and clinicopathological findings of 59 short- and long-term surviving dogs with PLE secondary to CE, and investigated potential prognostic factors. Consistent with previous observations, the adult dogs of any size were affected by PLE (Allenspach et al., 2007; Lecoindre et al., 2010; Dossin and Lavoue, 2011; Dandrieux et al., 2013; Simmerson et al., 2014), with a predominance of males, however (Kull et al., 2001; Simmerson et al., 2014). Medium size (11 to 20 kg) has recently been reported as a negative prognostic indicator (Equilino et al., 2015). When we compared the two groups, we observed that body weight was significantly higher

among the STs. This might simply reflect the type of study population or suggest that large breed dogs might be affected by more severe forms of PLE.

Small bowel diarrhea and decreased appetite were the most common historical complaints in both groups. A recent retrospective study found that vomiting was a negative prognostic factor (Simmerson et al., 2014), however, we noted no significant differences in the presenting complaints/clinical signs between the two groups at T0. As seen also in our sample, ascites or pleural effusion are common complaints or physical examination findings in dogs with PLE (Allenspach et al., 2007, Lecoindre et al., 2010) but they do not seem to be negative prognostic indicators (Simmerson et al., 2014). Activity indices for assessing disease severity can also be used as prognostic markers (Jergens et al., 2003; Allenspach et al., 2007). According to one study, CCECAI ≥12 at diagnosis predicted refractoriness to treatment and euthanasia within 3 years (Allenspach et al., 2007). To the contrary, in our and in a recent study (Equilino et al., 2015), outcome or survival time were not significantly influenced by activity indices at diagnosis.

The only significant difference in pathologic variables between the two groups at T0 was the blood urea nitrogen concentration, which can be influenced by dehydration, renal failure or severe GI protein loss. But because we had no information about prerenal and renal azotemia values in these PLE dogs, this result should be interpreted with caution. Furthermore, the retrospective design of the present study is an additional limitation. Several variables tested at T0 were not available at T1 for all dogs, and treatments were not strictly standardized. That said, collectively, our results may support the hypothesis that the severity of clinical signs and the majority of serum biochemistry and coagulation profile findings at diagnosis do not appear to correlate with outcome.

The prognosis for dogs with PLE in the current veterinary literature is guarded (Allenspach et al., 2007; Dossin and Lavoue, 2011). Except for a recent retrospective study (Simmerson et al., 2014), there are few reports of survival data for dogs with PLE (Craven et al., 2009; Simmerson et al., 2009; Dijkstra et al., 2010; Goodwin et al., 2011; Owens et al., 2011; Equilino et al., 2015). Although 32.2% of the dogs had died within 6 months of diagnosis, a greater proportion (55.9%) was still alive at the time of manuscript preparation, suggesting that not only PLE-affected Yorkshire Terriers, but also other PLE-affected breeds may experience remission of clinical signs and prolonged survival despite severity of clinicopathologic findings at diagnosis, as recently described (Equilino et al., 2015).

To our knowledge, no long-term data on the follow-up of dogs with PLE exist. At T1 (6 months follow-up) the CCECAI scores were higher and the albumin, total protein, and total cholesterol concentrations all lower in the STs. Moreover, the dogs with a CCECAI score > 5 were more likely to die within 6 months of initial diagnosis. Since these variables at T0 did not significantly influence the outcome, they might simply reflect a poor response to therapy. Finally, since all STs were categorized as immunosuppressive-unresponsive, it is reasonable to assume that a poor response to therapy is a poor prognostic indicator. Indeed, survival time was shorter in the dogs with high CCECAI scores at T1 and that were unresponsive to therapy.

In conclusion, the clinical outcomes of PLE are variable, with the majority of the dogs having prolonged survival despite the severity of clinicopathological findings at diagnosis

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CONCLUSION

Chronic GI signs are daily concerns for veterinarian patients in clinical practice. This thesis was intended to increase awareness among clinicians in the recognition of CE and assess the severity of this disease. Moreover, once the diagnosis is made, it is important for the patient's quality of life to choose the right treatment; from simple dietary changes for some dogs to important immunosuppression for others.

In conclusion, CE is a common disorder with variable prognosis from favorable to poor, in both dogs and humans. Unfortunately, even though GI disease is currently an intense area of scientific research, pathogenesis, diagnosis, and treatment are still not completely understood. For this reason, the study of CE is important for canine health and shares a common interest with human medicine.