UNIVERSIDADE DE LISBOA

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THE IMPACT OF DIET IN THE MANAGEMENT OF CANINE CHRONIC DIARRHOEA: FROM CLASSIC TO REFRACTORY CASES

SOFIA DUVERGÉ RODRIGUES

ORIENTADOR:
Doutor Rodolfo Assis Oliveira Leal

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O IMPACTO DA DIETA NA ABORDAGEM TERAPÊUTICA DE DIARREIA CRÓNICA EM CÃES: DOS CASOS CLÁSSICOS AOS REFRATÁRIOS

Resumo

O maneio alimentar é uma componente essencial em casos de diarreia crónica (DC). Geralmente, as dietas hidrolisadas são uma das primeiras abordagens terapêuticas com uma resposta satisfatória e bons resultados a longo prazo. Em casos de enteropatia crónica refratária ao tratamento (ECR), a utilidade de novas transições alimentares é questionável visto que, na maioria dos casos, as tentativas anteriores de maneio alimentar revelam-se infrutíferas. Este estudo subdividiu-se em duas partes e teve por objetivo: 1) avaliar a proporção de cães com DC que se apresentaram a consulta em centros de referência sem nunca terem feito um ensaio com dieta hidrolisada; 2) analisar o efeito de uma nova dieta alternativa em casos de ECR que já experimentaram anteriormente um ensaio dietético com dieta hidrolisada sem sucesso.

Foi efetuado um estudo retrospetivo que envolveu três centros de referência europeus, tendo sido revistos os registos médicos de cães com DC (> três semanas). Foram analisados dados relacionados com tratamentos de suporte, antibióticos e imunossupressores prescritos antes destas consultas. Cães com suspeita ou confirmação de doenças extra-digestivas foram excluídos. Os casos incluídos foram avaliados quanto ao historial alimentar e posteriormente caracterizados. Na população incluída foi efetuada uma seleção de cães com ECR, dos quais parte foram caracterizados de acordo com uma nova transição alimentar, pelo tipo de dieta implementada e melhoramento clínico.

Foram incluídos 142 cães, dos quais 57% (81/142) casos nunca tinham experimentado uma dieta hidrolisada anteriormente à consulta. Destes, 47% (38/81) não estavam com nenhuma medicação, 30% (24/81) estavam com tratamento de suporte, 12% (10/81) estavam sob antibióticos, e por fim 11% (9/81) estavam sob imunossupressores. Foi instaurada dieta hidrolisada em todos os 81 casos. Enquanto em 20% destes (16/81) a gestão dietética foi a única medida, nos restantes 80% (65/81) foram prescritos outros tratamentos concomitantes. Foi observada uma melhoria clínica em 70% (57/81). Um total de 26 casos (18.3% da amostra inicial) foram identificados como ECR. Destes, 88,5% (23/26) experimentaram uma nova transição alimentar. Em 69,4% (16/23) esta alteração foi exclusiva, sendo que 68,8% (11/16) melhoraram. A associação de dieta com tratamentos complementares foi realizada em 30,4% (7/23), tendo resultado numa resposta positiva em 42,9% (3/7) destes cães.

Este estudo realça a falta de prática de uma transição alimentar adequada antes de referência/consulta de 2ª opinião, e evidencia sua importância na linha de tratamento de DC. Além disso, salienta ainda que deve ser considerada uma nova dieta em casos de ECR.

Palavras-chave: diarreia crónica, canídeo, dieta hidrolisada, gestão dietética, enteropatia crónica refratária

THE IMPACT OF DIET IN THE MANAGEMENT OF CANINE CHRONIC DIARRHOEA: FROM CLASSIC TO REFRACTORY CASES

Abstract

Dietary management is one of the major components of canine chronic diarrhoea (CD) treatment. Hydrolysed diets are usually considered one of the therapy approaches with a satisfactory response and good long-term outcomes. In cases of canine refractory chronic enteropathy (RCE), the value of a new dietary transition is questionable considering that previous diet experiments were ineffective in most cases. This study was divided into two parts and aims to 1) evaluate the proportion of canine CD cases presented in referral centres in which a hydrolysed diet was not previously implemented, analysing the relevance of this diet; 2) assess the effect of a new dietary transition in canine RCE cases that have already been fed with a hydrolysed diet ineffectively.

A retrospective multicentric study was developed involving three european referral centres. Medical records of dogs presented with CD (>three weeks) were reviewed. Data regarding supportive treatments, antibiotics and immunosuppressants prescribed prior to these consultations were analysed. Dogs with a suspected or confirmed extragastrointestinal disease were excluded. The cases included were evaluated considering their dietary history and were further characterised. Also, a selection of RCE dogs was made among the population included. A diet transition was performed in a proportion of RCE dogs, which was then characterised according to dietary choice and clinical improvement.

In this study, 142 dogs were included, of which 57% (81/142) had never received a hydrolysed diet prior to consultation. Of these, a total of 47% (38/81) were not under medication, 30% (24/81) were on supportive therapy, 12% (10/81) were being administered antibiotics, and lastly, 11% (9/81) were on immunosuppressants. In all 81 dogs, a hydrolysed diet was introduced, in which 20% (16/81) diet was the solo treatment measure changed, and in the remaining 80% (65/81), other ancillary treatments were prescribed simultaneously. Overall, a clinical improvement was documented in 70% (57/81) of these dogs. A total of 26 dogs (18,3% of the initial population) were identified as RCE cases. Of the latter group, 88,5% (23/26) experimented another alimentary transition. In 69,4% (16/23) of these cases, diet modification was exclusive, in which 68,8% (11/16) showed a positive response. Association of the diet with ancillary therapy was only made in 30,4% (7/23), with a favourable response in 42,9% (3/7).

This study highlights the paucity of practising an appropriate dietary transition before referral or second opinion consultations and enhances its importance in the therapy line of CD. Furthermore, it emphasises that in cases of canine RCE, a new dietary transition should be considered.

Keywords: chronic diarrhoea, canine, hydrolysed diet, dietary management, refractory chronic enteropathy

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List of abbreviations

AB-Antibiotics

AEs-Antiemetics

alpha-P1-alpha1-protease inhibitor

ARD-Antibiotic-responsive diarrhoea

BUN-blood urea nitrogen

CCECAI-Canine Chronic Enteropathy clinical activity index

CD- Chronic Diarrhoea

CE-Chronic Enteropathy

CIBDAI-Canine Inflammatory Bowel Disease activity index

CRP-C-reactive protein

cTLI- canine serum trypsin-like immunoreactivity

e.g.- exempli gratia (Latin) (for example)

ECVIM- European College of Veterinary Internal Medicine

ELISA– enzyme-linked immunosorbent assay

EPI-Exocrine pancreatic insufficiency

FISH-Fluorescence in situ hybridisation

FMT-Faecal microbiota transplant

FNA- Fine-needle aspiration

FRE- Food-responsive enteropathy

FS-Fibre source

GC-Granulomatous colitis

GI-Gastrointestinal

GPs-Gastric protectants

HA-Hypoallergenic

HEV-FMV- Veterinary Teaching Hospital of the Faculty of Veterinary Medicine

IBD-Inflammatory bowel disease

IQR- Interquartile Range

IRE- Immunosuppressant-responsive enteropathy

LPE- Lymphoplasmacytic enteritis

p- probability

PAAR- Polymerase chain reaction for antigen receptor rearrangement

PAS- Periodic-Acid-Schiff

PAS-Periodic-Acid-Schiff

PBs-Probiotics

PCR- Polymerase chain reaction

PLE- Protein-losing enteropathy

PO- per os

q12h- every 12 hours

q24h- every 24 hours

q8h - every 8 hours

RC-Royal Canin

RCE- Refractory Chronic Enteropathy

SG1-Study Group 1

SG2-Study Group 2

SIBO- Small intestinal bacterial overgrowth

ULFD-Ultra-low-fat diet

US-Ultrasound

WSAVA-World Small Animal Veterinary Association

List of symbols and units

n-size of sample

%-percentage

®-registered trademark

= equal to

< less than

> more than

≥ more or equal

± more or less

g/L-gram per litre

µg/g- micrograms per gram

µg/kg-micrograms per kilogram

ng/L-nanogram per litre

mg/kg-milligram per kilogram

mg/m^2-milligram per square metre

Mcal-Megacalorie

ME-metabolizable energy

TDF- total dietary fibre

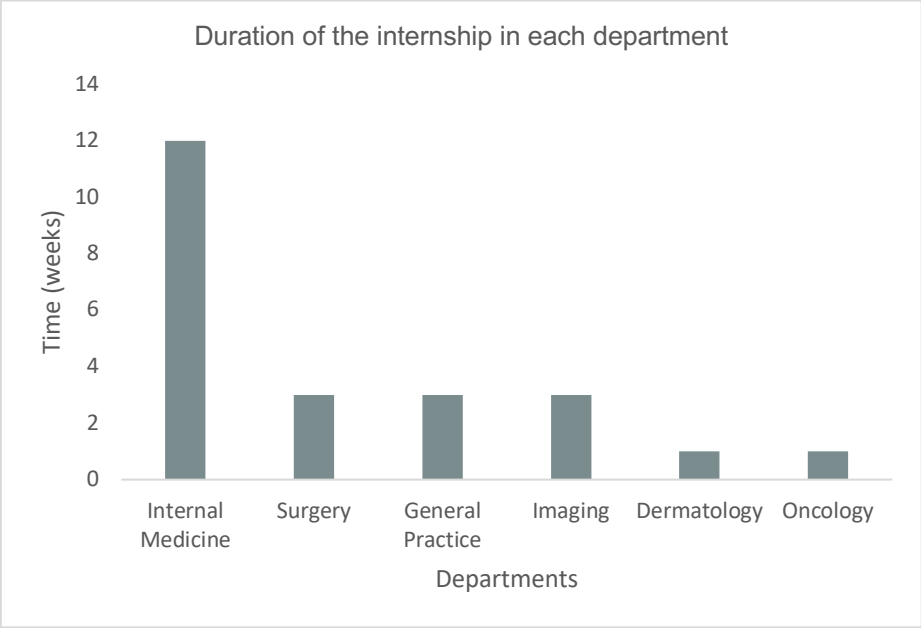
Section 1- TRAINEESHIP REPORT

1. Internship at HEV-FMV

This report describes the work performance accomplished during the curricular internship. The internship took place at the Veterinary Teaching Hospital of the Faculty of Veterinary Medicine (HEV-FMV)– The University of Lisbon from the September 6th 2021 to the February 7th 2022. The activities were achieved on a rotation basis across the multiple departments from the HEV-FMV, detailing Internal Medicine, Imaging, Dermatology, Oncology, Surgery and General Medicine. Hospitalisation and night shifts were also part of the rotations. All activities performed were supervised by a veterinary surgeon, veterinary nurse, or a technician. During the first 5 months, there was a weekly-basis *Bookclub* within the Internal Medicine Service, which consisted of presenting one chapter of the book Ettinger's '"Textbook of Veterinary Internal Medicine: Diseases of the Dog and the Cat"' to the other colleagues in this department.

The work hours and weeks spent in each service were variable, including a day or evening shift of 8 hours. A 12 twelve-hour night shift occurred twice a month during the internship period. Graphic 1 presents the weeks spent on each service regarding the various departments.

Graphic 1-Weeks spent in each department at HEV-FMV.



Internal Medicine

This department represented 3 months of the total of the internship. For most of the time in this department, the activities were supervised by Professor Rodolfo Leal, a board-certificated specialist in Internal Medicine responsible for this service and thesis advisor. Apart from Professor, Dra Joana Dias (Internal Medicine Resident) and Dr Nuno Santos (Speciality intern) were part of the team and also supervised some activities.

The day always started with the morning rounds, which began at 8.30 a.m. with some hospital staff from the various departments. This was focused on the hospitalised patients from the HEV-FMV and its different services, consisting of the presentation of the clinical cases by the night-shift clinician and discussion of diagnostic and therapeutic plans by all the medical staff.

Afterwards, referral, first and second opinion and re-evaluation consultations were attended during the morning. These consultations involved various subjects, such as gastroenterology, endocrinology, respiratory diseases, nephrology, and many others. Consultations attended were mainly led by Prof. Rodolfo, but when he was absent or to experience a higher range of different cases, appointments from the other practitioners of the team were followed. In these, it was given a chance to collect clinical history and complete anamnesis, perform physical exams and blood pressure measurements as well as blood sampling. Participation in discussing the diagnosis and treatment plan was always encouraged. This was extremely important to collect data from the consultation reports in order to achieve this work. Moreover, discussion of differential diagnosis, bloodwork results and therapeutic approaches from the morning cases were conducted in the afternoon.

Collaboration in other medical, complementary exams was possible, once or twice a week. The student could aid and observe upper and lower gastrointestinal endoscopies, bone marrow aspirations, synovial fluid aspirations, rhinoscopies, bronchoscopies with bronchoalveolar lavages and urethral and cystoscopies.

Surgery

In this department, the student remained for 3 weeks in an 8hour morning shift of elective and emergency surgeries. At 8.00, there was participation in the animal's admissions in the surgery service, where a pre-surgery checklist was discussed with the patient's owners. Then, sedation was prepared and administered for the tranquillisation of the patient, followed by all other pre-surgical procedures, such as peripheral venous catheterisation, pre-anaesthetic and induction drugs, asepsis, and tracheal intubation. Pre, peri, and post-surgical monitorisation of the animal was always part of the student's activities, as well as participation in the surgical procedures by observation or aiding the lead surgeon as an assistant. In addition, there was a discussion of the best anaesthetic and monitoring plans, post-surgical care, and the cases chosen for the following surgeries.

General Practice

The general practice rotation lasted approximately 3 weeks with 8-hour shifts and some extra hours. The shift usually started with the morning round in the inpatient care unit, when no consults were performed in the morning. Regardless of the time of the shift, this rotation mainly consisted of first-opinion consultation supervised by first-opinion veterinarians and prioritised emergency cases. The student was able to perform a complete anamnesis and physical exam on its own, including a neurological, orthopaedic, and dermatologic exam. After collection of the medical history by the student, a discussion between the student and the supervising clinician was followed regarding the main problems, diagnosis, and treatment plan. There was also the chance to participate in other procedures, such as collecting blood samples, inserting venous catheters, executing cystocentesis, skin or ear cytology, and quick ultrasounds, plus thoracocentesis and abdominocentesis.

Once a week, there was a 12-hour shift in the hospitalisation yard, in the morning, the aiding of the veterinary nurses was essential, where the student learnt to prepare and administer medications, collect blood, adjust fluid therapy plans, and help in the physical exams of the patients. In the afternoon, after the 15h round, there was time to discuss more diagnoses and treatment plans, alongside the reading of some articles concerning specific complex cases and making medical discharges. Besides medications, the student also had the chance to help with urinary catheter placement, blood transfusions, oxygen supplementation, oesophageal or nasogastric tube placements, thoracocentesis and abdominocentesis.

Imaging

During one week in 10-hour shifts, the student learned from the imaging department about radiographic and computed tomography (CT) exams. At 8h am, the CT reports were created in conjugation with the supervision of Dr António Almeida, a certificated holder diagnostic imaging practitioner. Then, throughout the day, the student assisted in the diagnostic imaging exams, with the animal's admission, insertion of venous catheter, sedation, intubation, anaesthesia monitorisation, reporting the situation to the owners and followed the recovery of the patients after these exams. During this time, the student improved positioning techniques as well as her interpretation of various tests and differential diagnoses. Other procedures were observed, such as cerebrospinal fluid collection and needle biopsies.

A period of 2 weeks was spent in observation and assistance of ultrasonographic examinations and participation in the exam reports. In addition, other procedures were aided, for instance, cystocentesis, ultrasound-guided percutaneous needle biopsies and Subcutaneous Ureteral Bypass (SUB) lavages.

Dermatology

The dermatology service accounted for one week rotation, in slightly 8 hours shifts. In these, there were mainly first-opinion and re-evaluation dermatology consultations, as well as second opinion consults. The student usually started by collecting a complete, physical exams and ear or skin cytology. There was always the opportunity to discuss the differential diagnosis and treatment options with the clinicians of the service which thereafter conducted the consultations. Besides the standard procedures, observation of skin biopsies and video otoscopies were also possible.

Oncology

The student passed throughout this department for one week, with 8-hour shifts. The student mainly followed first-opinion, second-opinion, and re-evaluation consultations. Alongside the standard procedures, chemotherapy sessions and aspiration biopsy by fine needle of nodules or masses were assisted.

Night shifts

Every two weeks, a night shift was included in the rotations. It started with the night medical round, with the presentation of the patients in the inpatient care unit and Infectious Diseases Isolation Unit (IDIU) by the day-time practitioners in the hospitalisation yard. The student assisted in the animal's clinical monitorisation, medication preparation, and administration in line with the doctor's plan established during the day and with the assistance of the night doctor and nurse. The student participated in all the current medical procedures either standard or emergencies.

Scientific communications

Two abstracts with the preliminary results of this study were submitted to the European Society of Comparative Gastroenterology Stream from the European College of Veterinary Internal Medicine – Companion Animals 32nd Annual Congress of 2022 in March. If chosen by the committee, they will be presented in September 2022 in Gotheburg, Sweden (Annexe 1 and 2).

Section 2- STUDY BACKGROUND

1. Chronic Diarrhoea

1.1. Definition, pathogenesis classification

Diarrhoea is a frequent complaint for consultation in dogs, resulting in severe morbidity and owner anxiety (Walker and McMahon 2019). This clinical sign arises from an excess of faecal water, which results from poor intestinal absorption or increased intestinal secretion (Willard 2017). There are four central pathophysiologic mechanisms involved in diarrhoea: 1) dysmotility, including cases of sudden diet changes, or secondary to inflammation; 2) osmotic, usually from malabsorption; 3) secretory, associated with chemical or bacterial toxins; and 4) permeability changes, normally due to inflammation or neoplastic infiltrates, consequently leading to an exudate. The above-mentioned mechanisms are frequently associated. For instance, intestinal inflammation might prompt dysmotility, poor absorption and permeability alteration (McCann and Simpson 2006; Hall and Day 2017).

There exist countless categorisation schemes for diarrhoea. The most common ones focus on diarrhoea duration (acute or chronic), anatomic location (extragastrointestinal versus gastrointestinal (GI): small-intestine or large-intestine diarrhoea) and pathophysiology (allergic, biochemical, infectious, inflammatory, neoplastic, vascular, or lymphatic) (Marks 2013; Hall and Day 2017).

Generally, chronic diarrhoea (CD) is defined as when clinical signs persist or are intermittently present for more than three weeks or do not improve after 14 days (Allenspach 2015; Hall and Day 2017; Willard 2017). When clinical signs last less than 14 days it is considered an acute case of diarrhoea (Marks 2013).

In this thesis, it was adopted a classification of diarrhoea based upon the duration of clinical signs (chronic disease) and the location of disease (extragastrointestinal *versus* GI).

1.2. Aetiology of Chronic Diarrhoea

1.2.1. Extragastrointestinal causes

Several secondary non-gastrointestinal causes are relevant in a diagnosis approach of CD (Pipan 2019). This includes pancreatic disease (recurrent acute or chronic pancreatitis, exocrine pancreatic insufficiency, pancreatic carcinoma); liver disease (hepatocellular failure, cholestasis); renal disease (uraemia, nephrotic syndrome); polysystemic infections (leptospirosis, infectious canine hepatitis, leishmaniosis); endocrine diseases (hypoadrenocorticism and more rarely hyperthyroidism); others, such as portal hypertension, autoimmune diseases, metastatic neoplasia, and iatrogenic causes (nonsteroidal anti-inflammatory drugs, antibiotics, cancer chemotherapeutics) (Hall and Day 2017; Pipan 2019).

1.2.2. Gastrointestinal causes

CD is one of the most prevalent signs of GI disease in dogs, including gastric illness (gastritis, achlorhydria) or intestinal disease, namely primary small and large intestinal diseases, which will be detailed below (Hall and Day 2017).

1.2.2.1. Primary small intestinal diseases

Adverse food reactions such as intolerance, indiscretion, or allergy are one of the primary small intestinal diseases which must be considered when a CD is present (Walker and McMahon 2021).

Infectious diseases are uncommon in chronic cases. Its occurrence is most anticipated in younger, immunocompromised, and shelter dogs (Hall and Day 2017). Among infectious causes, parasitic and protozoal infections are the most prevalent in CD (Walker and McMahon 2021). These include intestinal parasites, namely some helminths (*Toxocara canis; Trichuris spp.),* nematodes *(Strongyloides spp.),* hookworms *(Ancylostoma spp.*), coccidial parasites (*Cryptosporidium spp*.; *Isospora spp*.), and protozoal infection (*Giardia spp*.). The latter infection might be solely a secondary cause of CD in some senior dogs (Walker and McMahon 2019). Likewise, bacterial enteropathies are also more frequent in acute cases. Nonetheless, if suspected, bacterial infection should be considered, particularly *Clostridium spp.*, *Campylobacter spp.* and *Salmonella spp*. In addition, depending on geographic location, fungal, algal or rickettsial organisms can also lead to CD (Allenspach 2015; Hall and Day 2017; Pipan 2019).

Chronic enteropathy (CE) is a significant cause of CD in dogs (Walker and McMahon 2021). This term refers to a group of idiopathic diseases with a multifactorial aetiology characterised by chronic persistent or recurrent clinical GI signs (Allenspach and Mochel 2022). There are three main subdivisions of CE which can be retrospectively classified according to their response to stepwise treatment trials: food-responsive enteropathy (FRE), antibiotic-responsive diarrhoea (ARD) or dysbiosis, and immunosuppressant-responsive enteropathy (IRE) (Dandrieux, Martinez et al. 2019). As part of IRE, inflammatory bowel disease (IBD) term is used when a full workup has been performed to exclude all extragastrointestinal and other intestinal diseases, failed food and antibiotic trials, confirmation of inflammation by biopsy and require immunosuppressants (Dandrieux 2016). Previously, the term SIBO (''Small intestinal bacterial overgrowth'') was used to describe ARD. However, recently the term ''Small intestinal dysbiosis'' has been accepted as the correct term associated with ARD, which is defined as an alteration of the composition and richness of the intestinal microbiota (Kather et al. 2020). Furthermore, dogs with CE associated with low albumin or hypoproteinaemia are classified as protein-losing enteropathy (PLE) (Dandrieux 2016). The most frequent mechanisms associated with PLE are altered lymphatic drainage or increased mucosal permeability, leading to a decrease on plasma oncotic pressure. This can be caused by several diseases namely IBD,

primary intestinal lymphangiectasia, intestinal neoplasia, severe intestinal infection and hypoadrenocorticism. Many breeds including Yorkshire Terriers and Rottweilers seem predisposed to PLE (Table 1) (Hall and Day 2017; Walker and McMahon 2021).

Lymphangiectasia can be primary when associated with generalised alterations in the structure of lymphatic vessels, or secondary to obstruction or infiltration of lymphatic vessels by inflammation, fibrosis, neoplasia, obstruction of the thoracic duct, right heart failure or cardiac tamponade (Hall and Day 2017).

Neoplasia should be investigated, especially in middle-aged and older dogs. The large cell lymphoma, albeit rare, is the most frequent form in dogs with CD (Walker and McMahon 2021), whereas the small cell lymphoma is not clearly defined in this species (Kiupel et al. 2011). Other forms of neoplasia can be considered, such as adenocarcinoma, stromal tumours, and mast cell GI tumours (Walker and McMahon 2021).

Finally, structural conditions, for instance, chronic intussusception or chronic partial obstruction, can lead to CD (Walker and McMahon 2019).

1.2.2.2. Primary large intestinal diseases

One of the primary large intestinal diseases is idiopathic chronic colitis, which is essentially an extension of IBD. German Shepherds and Shar-pei dogs are generally predisposed to this disease. Another disease that can occur in the large intestine is neoplasia which is similar to those identified in the small intestine. However, in the large intestine, adenocarcinoma is more common than lymphoma. Furthermore, structural alterations such as caecolic intussusception, parasitic (*Ancylostoma spp.*; *Trichuris vulpis*), and bacterial infections (*Escherichia Coli*, associated to granulomatous colitis (GC), particularly in Boxers and French Bulldogs) can also induce large-bowel CD (Hall and Day 2017; Pipan 2019; Walker and McMahon 2019).

1.3. Diagnostic approach

Two important aims of the diagnostic approach of CD are the exclusion of extragastrointestinal, and infectious diseases, as well as the differentiation of the location of primary GI illness (Hall and Day 2017). From then on, the following exams should be followed to achieve a CE diagnosis since this is performed on a step-by-step approach, by exclusion (Dandrieux 2016).

1.3.1. History and Physical exam

Firstly, a complete and detailed history is essential before any diagnostic test (Matz and Guilford 2003). All dietary and medical history including vaccination status, deworming status, supplements, dietary indiscretions (Hall 2009), exposure to infectious agents (Marks 2013), kennel stays, and travel history should be included (Hall 2009; Marks 2013). Age might give

an indication of the diagnosis since young dogs are more predisposed to develop FRE (Dandrieux 2016) and *Giardia* infections (Pipan 2019). Also, various breeds have some GI disease predisposition (Marks 2013), which are described in Table 1.

Table 1-Breed predispositions of GI diseases

Breed	***Disease***
Basenji	LPE
Beagle	SCM, aminopeptidase-N deficiency
Border Collie	IBD, PLE, SCM
Boxer	GC, IBD (LPE, EE)
French Bulldogs	GC
German Shepherd	idiopathic ARD, IBD (LPE, EE), EPI
Giant Schnauzer	SCM
Irish setter	GSE
Lundehund	Lymphangiectasia
Retrievers	FRE
Rottweiler	IBD, PLE
Soft-coated wheaten terrier	PLE/nephropathy
Shar-pei	LPE, PLE
Weimaraner	IBD
Yorkshire Terrier	Lymphangiectasia, PLE
West Highland White Terrier	FRE

Legend: Adapted from Hall and Day (2017), and Walker and McMahon (2019). LPE-lymphoplasmacytic enteritis, IBD- Inflammatory Bowel Disease, PLE- Protein-losing Enteropathy, SCM-Selective cobalamin malabsorption, N-nitrogen, GC-Granulomatous colitis, EE-Eosinophilic enteritis, EPI- Exocrine pancreatic insufficiency, GSE-Gluten specific enteropathy, FRE-Food-responsive Enteropathy.

Characterisation of clinical signs is necessary to confirm CD and its severity, especially if there is involvement of weight loss or blood in faeces since generally these have a significant reserved prognosis (Hall and Day 2017). Clinical signs and faecal appearance described in Table 2 give some direction of the disease's nature, which helps on the diagnostic investigation and on the decision concerning the site of possible future biopsies as well as to specify treatment and diagnosis (Hall 2009).

Table 2- Differentiation of Small *versus* Large Bowel Diarrhoea

Signs	***Small intestine***	***Large intestine***
Faeces		
stool volume	large	small
mucous	usually absent	often present
blood	melena	hematochezia
fat	maybe present	absent
undigested food	occasionally	absent
Defecation		
tenesmus	rare	common
frequency	2-3/day	>3/day
urgency	uncommon	common
dyschezia	absent	often present
Other signs		
vomiting	sometimes	uncommon
weight loss	common	uncommon
gas	common	rare

Legend: Adapted from: Marks (2013), and Hall and Day (2017).

A thorough physical exam is crucial to determine the origin and severity of the disease, as well as to exclude other systemic diseases (Hall and Day 2017). Low body condition score supports a possible malnutrition, which in the context of CD can be caused by IBD or intestinal lymphoma. Another relevant finding is fever which may suggest an infectious disease. Also, the mucosa membrane pallor might be related to anaemia due to intestinal bleeding and peripheral oedema may indicate PLE (Marks 2013). Furthermore, careful abdominal palpation can lead to the suspicion of ascites, assess pain, masses, foreign bodies, and changes in other organs, such as kidney or liver (Hall 2009; Marks 2013).

Other specific observations include a cutaneous examination to assess malnutrition or food hypersensitivity (Hall and Day 2017). In addition, it is imperative to perform digital rectal palpation to confirm diarrhoea (Marks 2013), search for masses or polyps, especially if the dog presents tenesmus, and collect samples for faecal examinations (Hall and Day 2017).

In cases of CD, the physical exam is occasionally normal, or with discrete abnormalities. However, it might be relevant to search for extragastrointestinal causes or comorbidities, which are not uncommon in canine CD (Walker and McMahon 2019) .

1.3.2. Laboratory tests and findings

The ideal approach is to perform a complete blood count, serum biochemistry and urine analysis (Hall and Day 2017). These exams are relevant to rule out extragastrointestinal causes of diarrhoea described above (Hall 2009). Also, electrolytes assessment might be extremely helpful in identifying hyperkalaemia and hyponatraemia, present in cases of hypoadrenocorticism (Schofield et al. 2021), *Trichuris vulpis* and *Salmonellosis spp.* infections (Hall and Day 2017).

Concerning hematologic findings, generally in chronic cases, packed cell volume is normal. Nevertheless, anaemia could be observed due to GI inflammation, neoplasia (which typically is mild non-regenerative anaemia) (Hall 2009), or from intestinal blood loss (more specifically a non-regenerative normocytic and normochromic anaemia) (Walker and McMahon 2019). Concerning white blood cell count and differential, eosinophilia can be present in case of endoparasites infection, eosinophilic enteritis, hypoadrenocorticism (Marks 2013), dietary hypersensitive, and macrocytic disease cases secondary to lymphoma. Additionally, leucocytosis is common in cases of inflammation, neoplastic, or infectious disease (Walker and McMahon 2019). Also, peripheral neutrophilia can be found in stressful situations, inflammation or infection, and lymphopenia might be detected in lymphangiectasia cases (Marks 2013).

On biochemistry profile, creatinine and BUN serum concentrations help on the detection of renal disease, and the latter parameter, when increased, can also indicate GI

haemorrhage (Palm 2017). A decrease in serum albumin concentration (<2 g/L) is correlated with a negative prognostic factor in dogs with IBD (Allenspach 2015). Hypoproteinaemia is a common finding in PLE cases (Marks 2013), though it might be present in liver failure and renal disease. To assist in prioritising the source of the panhypoproteinaemia, cholesterol is an important parameter, since most PLE and hepatic insufficiencies induce hypocholesterolaemia. In contrast, in nephropathies, it is typically accompanied by hypercholesterolemia (Matz and Guilford 2003; Hall and Day 2017). Furthermore, in dogs with PLE and intestinal lymphoma lower values of vitamin D concentration have been reported, as well as hypocalcaemia and hypomagnesaemia (Gow et al. 2011; Dandrieux 2016). Lastly, an elevation of hepatic enzymes should be carefully analysed because a mild increase can be explained by GI inflammation (Marks 2013) which can be justified by the reactivity hepatopathy involving bacteria, endotoxins, and cytokines that damage via portal circulation (Marks 2013).

Depending upon the situation, a specific canine Pancreatic Lipase Immunoreactivity (cPLI), and concentration of basal cortisol, following a corticotropin (ACTH) stimulation test, exclude more accurately pancreatitis and hypoadrenocorticism, respectively (Allenspach 2015; Pipan 2019).

1.3.3. Faecal examinations

A faecal examination is essential to identify the most common canine endoparasites (Hall and Day 2017).

Rectal cytology might be relevant for diagnosing inflammatory, infectious, and neoplastic diseases in dogs (Matz and Guilford 2003). However, the results are frequently negative (Hall and Day 2017).

A direct smear is suitable for an initial screening for ova, oocysts, larvae, trophozoites of protozoal parasites or nematodes. This includes *Giardia spp*. cysts and trophozoites, but false negatives are frequent (Walker and McMahon 2019), as well as *Isospora spp.* oocysts (Pipan 2019) and *Strongyloides stercoralis* larvae (Matz and Guilford 2003). When stained on the direct smear, finding three or more spores of *Clostridium perfringens* per high-power field can help diagnose enterotoxigenic-associated disease. If the presence of slender or curved rods in the shape of an S are detected, it can be suggestive of a *Campylobacter*-like bacteria (Pipan 2019). In addition, the modified Ziehl-Neelsen oocyst stain technique could be used as an initial screen test for *Cryptosporidium* oocysts*,* although it is not a sensitive test for small animals (Scorza and Tangtrongsup 2010). The mentioned diagnosis should be confirmed with other tests since there is a small sample size in a direct smear and no parasite concentration (Matz and Guilford 2003).

To identify and search for GI parasites, such as helminth eggs, protozoan cysts or oocysts, faecal concentration techniques are the conventional methods used (Sobotyk et al.

2021). The zinc sulphate flotation test is the chosen method to identify *Trichuris* species and *Giardia* oocysts. It is recommended to perform three consecutive tests since it possesses sensitivity of 95%. (Hall 2009; Pipan 2019). Sedimentation, or the Baermann technique, is also useful for detecting Strongyloides' larvae (Hall and Day 2017).

Faecal cultures are indicated in dogs with haemorrhagic diarrhoea, fever, inflammatory leukogram or when neutrophils are identified in rectal cytology. Some bacteria can be isolated, for instance, *Salmonella spp.*, *Campylobacter jejuni*, *Clostridium diffficie, Clostridium perfringens, Yersinia spp.* and some fungi, including *Histoplasma capsulatum* (Hall and Day 2017). Many bacterial species isolated on faecal culture can also be found in the faeces of healthy dogs, therefore, the utility of this test is debatable (Walker and McMahon 2019; Werner et al. 2021).

PCR test has the same limitations as faecal cultures. It is usually performed when there is a great suspicion of an infectious cause or when multiple dogs are affected (Walker and McMahon 2019). This technique is one of the most common methods to detect *Escherichia coli*, associated with several diseases, namely GC in Boxer and French Bulldogs (Marks 2017; Pipan 2019), being the only CE with bacteriologic aetiology confirmation. Nonetheless, it is worth mentioning that when GC is suspected, further investigation, including PAS staining and FISH, is recommended to confirm the diagnosis (Hall 2017).

For a more accurate diagnosis of *Clostridium perfringens*-associated diarrhoea, an ELISA for *Clostridium perfringens* enterotoxin should be combined with a PCR to detect enterotoxigenic strains. The choice test is ELISA to detect toxins A and B from *Clostridium difficile* (Marks et al. 2011; Pipan 2019). Also, PCR distinguishes the species of *Campylobacter spp.*, following a positive culture; these are much more sensitive and reliable than faecal cultures (Hall 2017). Moreover, for *Cryptosporidium* and *Giardia*, an immunofluorescent antibody staining of a faecal smear is the most sensitive and specific (>90%) diagnosis test (Rishniw et al. 2010, Hall and Day 2017). The latter microorganism can also be identified in immunochromatographic tests (ELISA), Witness or Snap (Hall 2017), with good specificity, however, sensitivity is more variable (Dryden et al. 2006).

1.3.4. Faecal markers

The alpha1-protease inhibitor (alpha-P1) is a plasma protein of similar size as albumin. When rupture of the intestinal mucosa barrier occurs, alpha-P1 is lost at the same rate as albumin. The difference between these two proteins relies on the proteinase inhibitor properties, protecting the alfa-P1 from proteases, allowing its measurement in dog faeces (Allenspach 2015). On that account, an elevated concentration of faecal alpha-P1 is clinically beneficial as a GI marker of protein loss, enabling an early diagnosis of PLE (Heilmann and Steiner 2018), and distinction from other protein-losing disorders (Matz and Guilford 2003;

Allenspach 2015). However, from the author's knowledge, this test is only available in the United States of America through the Gastrointestinal Laboratory at Texas A&M University.

An immunoassay that measures the faecal calprotectin is another biomarker to consider, which correlates with intestinal inflammation and degree of histologic change. According to Heilmann et al. (2018), a faecal concentration of this marker ≥15,2 µg/g can distinguish animals with IRE from FRE or ARD with moderate sensitivity (37%) and high specificity (100%). Furthermore, some tests with low specificity are available to detect occult blood from ulcerated mucosa or malignant tumours (Hall and Day 2017).

1.3.5. Other diagnosis tests and markers

Over the years, several indirect tests have been developed to assess intestinal function (Hall 2009). In dogs with a suspected chronic small intestinal disease suggestive of malassimilation, the following step should be the confirmation of maldigestion or malabsorption. Exocrine pancreatic insufficiency (EPI) is common in dogs with maldigestion, thus it is crucial to perform a cTLI test in order to exclude this hypothesis since this is the most sensitive and specific test for EPI. When ruled out, malabsorption is diagnosed by exclusion (Willard 2017), although an intestinal biopsy must be performed to obtain a definitive diagnosis (Hall and Day 2017).

The breath hydrogen test evaluates bacterial metabolism within the GI tract. This test measures the hydrogen produced exclusively by bacteria when carbohydrate is metabolised, consequently, there is an increase of hydrogen in case of malabsorption of carbohydrates. (Matz and Guilford 2003; Hall and Day 2017).

Moreover, serum cobalamin and folate concentrations are helpful to assess malabsorption. They are water-soluble vitamins which are absorbed in different portions of the small intestine. On that premise, a decrease in folates may indicate malabsorption in the proximal intestine while a reduction in cobalamin can imply disease in the distal intestine. There are several conditions related to suboptimal levels of cobalamin in dogs, such as EPI, considering that the intrinsic factor is necessary for the absorption of cobalamin, which is produced mainly by the pancreas. Other conditions associated are chronic severe diseases including IBD, alimentary lymphoma, GI histoplasmosis, small intestine dysbiosis; and lastly inherited conditions (Kather et al. 2020). This is a critical GI disease marker since severe hypocobalaminaemia is associated with a negative prognosis (Volkmann et al. 2017). Recently, hypercobalaminaemia has been investigated and has risen some attention. In a study conducted by Kather et al. (2020), 48% (10/21) of hypercobalaminaemic dogs showed clinical signs of chronic GI disease. Along with the research led by Riz et al. (2021), in which most of the hypercobalaminaemic dogs were diagnosed with GI illness (57%-26/47).

Folates are used indirectly to evaluate dysbiosis (Marks 2013). Previously, it was suggested that an increase in the number of bacteria would be reflected in a rise of the folates' level since many bacteria produce them. Also, it was stated that a decrease in cobalamin levels was justified by the bacterial competition for a substrate. However, recent findings argue that this is an insensitive and non-specific test for small intestine dysbiosis, therefore, it is not the recommended test or justification for any antibiotic treatment (Kather et al. 2020). On this sense, the dysbiosis index has been studied nowadays, which is composed by a quantitative PCR of several bacterial species that are often modified in CE. This score may measure overall dysbiosis and, when used after therapy, can imply whether the microbiome has recovered (Allenspach and Mochel 2022).

A great number of inflammation markers have been developed, which might be relevant when evaluating a CE case. Nevertheless, most of the mentioned markers are still non-specific for diagnosis, prognosis, and monitoring progression. C-reactive protein (CRP) is a sensitive marker, and its quantity level can signal the presence of inflammation and guide the therapy response (Walker and McMahon 2019). A correlation between CRP and the canine IBD activity index (CIBDAI) is not consensual since Jergens et al. (2003) have reported this association, whereas Otoni et al. (2018) demonstrated a poor correlation between these two and showcased that CRP and histopathologic severity score were not correlated. In addition, Heilmann and Steiner (2018) argued CRP is used to monitor therapeutic efficiency and found a correlation of this marker with the canine chronic enteropathy clinical activity index (CCEAI). Serum and faecal calprotectin, S100A12, 3-bromotyrosine, N-methylhistamine and perinuclear antineutrophilic cytoplasmic antibodies are other markers that still lack investigation on their efficiency and utility (Westermarck 2016; Walker and McMahon 2019)

1.3.6. More advanced diagnosis tests

1.3.6.1. Diagnosis imaging

Plain radiographs are a limited diagnosis procedure in dogs with CD (Walker and McMahon 2019). These are more advantageous when the goal is to either detect surgical emergencies (masses, intussusceptions, obstructions, foreign bodies, free peritoneal gas, intestinal placement), ileus, decreased serosal detail, pleural effusions or changes associated with histoplasmosis (Hall and Day 2017; Walker and McMahon 2019).

Ultrasound (US) has a higher sensitivity than radiography (Gaschen L 2011; Hall and Day 2017) and carries a good specificity in detecting masses, intussusceptions, foreign bodies, intestinal wall thickening and lymphadenopathy. The last two findings can be present in chronic inflammatory, lymphatic, and neoplastic enteropathies (Hall and Day 2017). Also, it is helpful to rule out extragastrointestinal causes of diarrhoea (Hall 2009).

An increase in intestinal wall thickness (Lecoindre et al. 2010) and alteration of wall layers echogenicity in the US, have been described in some canine diarrhoea cases (Kull et al. 2001; Penninck et al. 2003; Sutherland-Smith et al. 2007; Gaschen L et al. 2008; Lecoindre et al. 2010; Bota et al. 2016; Mapletoft et al. 2018). In addition, Gaschen L et al. (2008) argued that the mucosal echogenicity could be a more precise indicator of IBD than intestinal wall thickness in cases of CD in dogs. Additionally, the identification of hyperechoic lines in the jejunum demonstrated a sensibility of 75% and a 96% specificity in cases of PLE in dogs (Gaschen FP 2013; Peterson and Willard 2003). The abdominal US can also be valuable in choosing the best intestinal biopsy method and enables fine-needle aspirations or biopsies (Walker and McMahon 2019). Despite these reports, a recent study showed that veterinarians affirmed that in 53% of the dogs which performed an abdominal US, this technique had no additional utility in the diagnosis, putting into question its routinely use in cases of CD (Mapletoft et al. 2018).

1.3.6.2. Intestinal Biopsies

Previously, after excluding dietary, parasitic, systemic, or metabolic diseases, the investigation would move forward towards infectious causes and antibiotic trials, being intestinal biopsies and histopathology the latter processes. Recently, Cerquetella et al. (2020) proposed a novel approach to rational the use of antibiotics in cases of CD, recommending the execution of intestinal biopsies following diet trial and exclusion of the various diseases mentioned above. Subsequently, if no significant cellular infiltrate is found on histopathology, antibiotic trials specific to certain circumstances are implemented, which are described further in the therapeutic line.

The most common methods to perform an intestinal biopsy are endoscopically or surgically (Walker and McMahon 2019). Endoscopy is safer and faster than surgery and it allows the finding of focal mucosal lesions that would not be found in surgery considering that only the serosal surface is visible (Willard 2017). Also, it enables the collection of several tissue biopsies from different locations; diagnosis of selected lesions and it carries less risk of perforation and septic peritonitis compared to surgery (Washabau et al. 2010). In addition, colonic biopsy should be performed endoscopically because there is a higher risk of peritonitis and dehiscence when executing full-thickness incisions on the colon (Willard 2017). Surgery can be useful when dense submucosa or muscular involvement is suspected and when the alteration is out of endoscopic reach (Hall and Day 2017). Other circumstances when surgery can be advantageous are suspected lymphoma or IBD cases, in which a full-thickness biopsy should be performed since the histological changes may be indistinguishable in superficial mucosal biopsies from endoscopy (Washabau et al. 2010). Moreover, ileum biopsies have been demonstrated to be of extreme importance in certain diseases (lymphoma, IBD,

lymphangiectasia) to achieve a definitive diagnosis therefore, it is recommended, when feasible, to always attempt an ileal biopsy (Washabau et al. 2010; Hall and Day 2017).

1.3.6.3. Histopathology and complements

Histopathology of intestinal biopsies is challenging to interpret, and it possesses relevant limitations. The World Small Animal Veterinary Association (WSAVA) GI Standardization Group developed histopathologic scoring schemes and standardised criteria since there were disagreements between histopathologists when interpreting endoscopically biopsies (Hall and Day 2017). They suggested that microarchitectural alterations had a higher relevance in the diagnosis of CE in dogs than cellular infiltrates (Washabau et al. 2010; Allenspach, Mochel, Du et al. 2019). However, there was still a very high interpathologist variability with these new schemes in one retrospective study (Willard and Mansell 2011).

CE holds a great variety of histopathological presentations (Wennogle et al. 2017). Lymphoplasmacytic inflammation is the prevailing cellular infiltration in CE and IBD, but it does not forecast which therapeutic modality each dog will respond to (Dandrieux 2016). Also, it might be present a total or partial villous atrophy, as well as abscesses in the Lieberkühn crypts. Eosinophilic inflammation is the second most common infiltrate (Hall and Day 2017), which can be concurrent to lymphoplasmacytic inflammation, confirming the hypothesis of a multifactorial aetiology of CE in dogs (Allenspach and Mochel 2022). Additionally, severe eosinophilic infiltration can be related to erosion, ulceration, or spontaneous intestinal perforation associated with melena or haematochezia (Walker and McMahon 2021). Dogs with FRE has reports of eosinophilic infiltrates, however, this is not considered enough for diagnosis. Another cellular infiltrate which could be present in dogs with CE is neutrophilic or granulomatous infiltrate that might imply an infectious aetiology (Dandrieux 2016). Lastly, hypoalbuminaemia, dilatation of lymphatic vessels or lesions on intestinal crypts were frequently reported in PLE, as well as the presence of mucous on the crypts which has been associated with Yorkshire Terriers (Simmerson et al. 2014; Hall and Day 2017)

Other relevant findings related to histopathology were the lack of correlation between intestinal histopathology and the severity of clinical signs, serum and faecal biomarkers (CRP, canine faecal calprotectin) or the response to treatment in dogs with IBD (Willard and Mansell 2011; Westermarck 2016; Wennogle et. al 2017; Otoni et al. 2018).

Generally, in cases of ARD, motility disorders, undiagnosed EPI, dietary indiscretion, intestinal sclerosis, secretory diarrhoea and FRE, histopathology does not reveal significant alterations (Hall 2009; Willard 2017).

Some adjuvant techniques to histopathology can diagnose specific diseases and are essential to help distinguish intestinal lymphoma from severe lymphoplasmacytic enteritis. Immunophenotyping can be executed by fine-needle aspiration (FNA) samples using flow

cytometry, immunohistochemistry on biopsies, PAAR clonality testing or immunocytochemistry on FNA (Villiers 2020). In the last-mentioned technique, some T- or B-cell markers are available, however, there are fewer usable antibodies, and turnaround time is slower than the other methods available, such as flow cytometry and PAAR. The flow cytometry is advisable considering its higher sensitivity in immunophenotyping and contribution to additional prognostic information, for instance, MHCII expression in a high-grade B or T cell lymphoma is an indicator of prognostic. For lymphomas, numerous antibodies are accessible, but the panel should always incorporate two or more lineage-specific markers, besides the MHCII (Villiers 2020). The PAAR clonality testing amplifies the T-cell and B-cell antigen receptor genes and detects the presence of a clonally expanded population of lymphocytes (Allenspach 2015). A positive clonality test suggests a lymphoma diagnosis and can exclude a reactive lymphocytosis. This test has a high specificity for neoplasia, which is advantageous in indolent lymphoma or leukaemia (Villiers 2020). Although, false positives have been registered in cases of hepatitis associated with drug hypersensitivity, ehrlichiosis, leishmaniosis, and a regressing histiocytoma (Keller et al. 2016; Villiers 2020). In addition, barring the B-cell *versus* T-cell information, no additional prognostic indicators are present on the PAAR results (Villiers 2020). Immunohistochemistry is also useful on intestinal biopsies for diagnosing intestinal lymphoma and classifying its subtype (Hall and Day 2017).

Moreover, in dogs with suspected GC, endoscopy should be contemplated earlier compared to other CD cases. PAS staining demonstrates PAS-positive macrophages, supporting the GC diagnosis. Equally important, the FISH technique is a highly sensitive recent method that identifies invasive bacteria, including *Escherichia coli*, in the intestinal biopsies (Dandrieux 2016; Hall 2017).

1.4. Therapeutic approach and management

1.4.1. Adjunctive and supportive treatment

Fluid therapy may be beneficial in cases of dehydration and electrolytic imbalance. Also, medical adjuvant drugs can be administered to improve other clinical signs, such as antiemetics and gastric protectants (Hall and Day 2017).

In cases of hypocobalaminaemia, supplementation is essential, as a better response to treatment is observed in CE and in intestinal neoplasia (Walker and McMahon 2019). Also, there are considerable metabolic changes that consequently lead to loss of body condition, inappetence and decline of intestinal disease (Hall and Day 2017). Hypocobalaminaemic dogs should be supplemented with either weekly parental (SC or IM) injections of 50µg/kg over 6 weeks followed by another injection one month later, or a daily oral dose of 50µg/kg over 12 weeks. This should be followed by a recheck of cobalamin levels one month after the last dose

and after supplementation discontinuation. Both administrations seem to be equally effective. (Kather et al. 2020; Walker and McMahon 2021).

1.4.2. Specific therapies

All infectious diseases should be addressed in concurrence with the agent identified or suspected, using specific antimicrobials or parasiticides (Hall and Day 2017). In particular, GC cases have a good response to enrofloxacin (5mg/kg PO q24h for 8 weeks), although there have already been reports of resistance (Craven et al. 2010; Manchester et al. 2013).

Equally significant, all extragastrointestinal, mechanical and neoplasia diagnosed must be treated accordingly to the cause in place. For instance, surgery in cases of obstructions (Pipan 2019) or chemotherapy in some intestinal lymphomas, especially the low-grade alimentary lymphoma, which has a high response and good survival time (Walker and McMahon 2021).

1.4.3. Empirical treatment

Generally, sequential empirical treatments are established, considering financial circumstances and no identification of a specific cause (idiopathic CD) (German et al. 2003; Hall and Day 2017). This includes deworming and dietary trials, usually implemented before invasive diagnostic approaches such as endoscopy (Cerquetella et al. 2020), since this might be enough and successful in most cases of CD (Volkmann et al. 2017). Antibiotics are currently recommended after these trials and histopathology, as well as immunosuppressants which are conventionally implemented after biopsy confirmation (Cerquetella et al. 2020).

1.4.3.1. Deworming

Before empirical treatment, it is essential to exclude or treat occult and intermittent parasitism with parasiticides such as fenbendazole (50mg/kg) for 3 to 5 days, regardless of faecal examinations (Walker and McMahon 2019).

1.4.3.2. Diet trial and dietary management: what do we know?

Dietary management is a crucial treatment step in canine CD (Hall and Day 2017). A dietary trial should be the first step in diagnosing and treating these cases, being systematically recommended, and used by clinicians (Makielski et al. 2019). This should be done after treatment for intestinal parasites and always before intestinal biopsies (Walker and McMahon 2021) in dogs that are eating and not hypoproteinaemic or following confirmation of intestinal inflammation with histopathology (Hall and Day 2017). On the other hand, PLE and other severer cases, usually perform endoscopy concurrently to dietary alteration and other aggressive treatments, as they have a poor prognostic (Dossin and Lavoue 2011; Dandrieux 2016).

Several GI diseases respond to dietary modification. These include adverse food reactions, CE, FRE, chronic gastritis, IPE, gastroesophageal reflux, pancreatitis, portosystemic shunt, dysbiosis and lymphangiectasis (Hall and Day 2017). It can be a vital adjuvant to treatment in most forms of CE (Jergens and Simpson 2012). Concerning adverse food reactions, they can have an immunological response (allergies, usually associated with pruritis) or a non-immunological response (food intolerance and indiscretion). FRE covers these food-related causes that respond to an exclusion diet trial since they have similar clinical presentations, diagnoses, and treatment approaches, regardless of their etiopathogenesis (Gaschen FP and Merchant 2011; Hall and Day 2017).

Indeed, FRE is one of the most diagnosed causes of CD (Walker and McMahon 2021), which typically affects younger dogs (Dandrieux 2016).

1.4.3.2.1. Impact of diet and nutrition on the GIT

Nutrition has a significant impact on the functions and components of the GI tract, including modulation of microbiota, effect on the mucosal immune system and on motility, and enhancement of epithelial barrier function, which have been imbalanced in several GI diseases, principally in CE (Kathrani 2021).

Moreover, modifications in intestinal microbiota when a dietary trial is introduced have been reported (Kathrani 2021). One study demonstrated a relevant increase in faecal microbiota richness with an extruded animal protein-free diet in a diseased dog. However, this was not observed in healthy dogs (Bresciani et al. 2018), suggesting that diet can modify intestinal functions in dogs with CE (Kathrani 2021). Another investigation concluded that dogs with a hydrolysed diet achieved clinical remission associated with changes in the intestinal microbiota composition, a decline in the number of pathobionts, and a reduction in the severity of dysbiosis (Wang et al. 2019). Moreover, the different microbial pattern's dependence on diet macronutrients reflects the flexibility of the GI microbiota in adapting its metabolic capacity according to the diet substrates available (Isidori et al. 2022). In addition, it is known that several nutrients, such as fat, amino acids, and vitamins (A and D), directly impact the GI mucosal system. There are few studies regarding this, although one study demonstrated a decrease in the mean duodenal lamina mononuclear cell score and the duodenal lamina propria densities of eosinophils mononuclear cells in dogs with CE after 6 weeks with a hydrolysed diet (Walker et al. 2013). Also, in terms of immunology, proteins consist of most food antigens, hence a new source of protein or hydrolysed protein should be emphasised to reduce diet antigenicity and consequently reduce intestinal inflammation (Isidori et al. 2022).

1.4.3.2.2. Diet trial

There is a scientific consensus that an exclusion diet should be introduced (Tørnqvist-Johnsen et al. 2020). In general, this diet consists of a commercial diet with a single protein source, hydrolysed diet, or a homemade diet exclusively (Walker and McMahon 2019). But there are several options available to treat CD, including a highly digestible, fibre-enriched, low fat, novel, hydrolysed, home-made and limited ingredient protein diets (Tolbert et al. 2022). However, the most commercially used diets in the treatment of CE in dogs are hydrolysed and single or novel source protein diets due to their success (Tørnqvist-Johnsen et al. 2020) associated with a longer period of remission (Makielski et al. 2019).

To take the most benefit of dietary strategies in CE, an individualised therapy with attention to the environment, as well as possible genetic susceptibility would be the ideal. However, trial and error with various elimination diets are the most common approaches, which remain effective (Kathrani 2021). A good clinical response is frequently observed after two weeks with the new dietary regimen exclusively (Allenspach, Culverwell et al. 2016; Walker and McMahon 2021). Moreover, there are situations when the owner prefers to feed other food alternatives or return to the previous diet after the trial. Thus, if there is an excellent response to the trial, it is recommended to remain for 12 weeks ideally, on a strict dietary regimen. This is to ensure that a clinical response is observed, as well as improvement of intestinal inflammation (Dandrieux 2016; Hall and Day 2017).

Nevertheless, to establish whether the diet response is due to eliminating hypersensitivity (removed or altered protein antigens), a diet rechallenge with the original diet should be performed after those 12 weeks (Dandrieux, Martinez et al. 2019), if they relapse one has a presumptive diagnosis of food allergy (Rudinsky et al. 2018). This is usually not implemented when animals are clinically stable, since it is time-consuming and unpractical for dog owners (Dandrieux, Martinez et al. 2019). Even though the gold standard test to diagnose adverse food reactions is still an elimination diet with subsequent provocations, a negative patch test with food ingredients has shown a very high negative predictability in dogs, and it could be valuable in selected dogs to decide which ingredients should compose the elimination diet (Mueller and Olivry 2017).

1.4.3.2.3. Different types of diets in the management of chronic diarrhoea

Highly digestible diets with low residue are usually considered the first choice for dogs with mild signs of small intestinal disease, without cutaneous signs of a food allergy and normal blood work (Tolbert et al. 2022). A highly digestible food reduces the intestinal antigenic load and substrates for the microbiota (Isidori et al. 2022). These diets are characterised by low fibre content, typically <10gTDF/Mcl, and use a highly digestible source of starches. The fat content varies depending on the commercial brand, hence the choice of this type of diet should

attend to the current fat intake and individual needs (Tolbert et al. 2022). Dogs that fail these diets should experiment a hydrolysed or novel protein source diet, before other medications (Mandigers et al. 2010). In cases of ARD, a highly digestible diet with low fat quantity decreases the substrates available for bacteria which can be beneficial (Ziese and Suchodolski 2021).

Proteins consist of most food antigens, hence a new source of protein or a hydrolysed protein diet should be emphasised in order to reduce diet antigenicity and consequently decrease intestinal inflammation (Isidori et al. 2022; Tolbert et al. 2022). This is achieved via enzymatic hydrolysis, which reduces protein structure into small polypeptide fragments (Rudinsky et al. 2018). A total disappearance of protein antigenicity is almost impossible to achieve, thus a hydrolysed diet with a novel protein the dog never had contact with before is preferable compared to one they possibly had been sensitive to (Cave 2006). Also, hydrolysed diets are generally of high digestibility compared to their source and have a lower fibre content. However, the hydrolysis may have effects on osmotic balance in the GIT and on palatability (Rudinsky et al. 2018). In general, both hydrolysed and novel sources of protein show improvement in clinical signs (Allenspach, Culverwell et al. 2016). On this account, the choice relies on the acceptance by the dog and on its individual nutrient needs (fat or fibre) (Tolbert et al. 2022). Concerning over the counter novel protein diets, these are not usually recommended considering cross-contamination with other animal proteins that are not labelled (Pagani et al. 2018).

Homemade diets are frequently implemented in severe CE and RCE cases, as food reactions and identical diets can lead to less appetite in these cases (Torres et al. 2003). A home-prepared meal can be used to create an ultra-low-fat diet (ULFD) (<17 g of fat/Mcal ME) or a diet with novel ingredients (Rudnsky et al. 2018). Also, it is simpler when commercial dietetic options do not complete all the nutrients needed for the dog, as well as minimise the risk of cross-contamination of undeclared protein in commercial diets. Furthermore, the cooking of these meals can increase digestibility and amino acid availability, though it might create new food antigens when cooked under extreme heat. Another point to take into account is that the amino acid composition and digestibility of different sources (plants, insects or exotic meals) differ widely, consequently, macronutrient and micronutrient deficiencies can be associated with homemade diets (Tolbert et al. 2022). Therefore, consultation and follow-up with a board-certified veterinary nutritionist are crucial (Walker and McMahon 2019; Tolbert et al. 2022).

Regarding lymphangiectasis and idiopathic PLE, the latest study suggests the most appropriate diet is a low-fat (<30g fat/Mcal ME) (Rudinsky et al. 2017), high-digestible diet with significant calories as most cases have a decreased body condition (Hall and Day 2017). They can also privilege from a novel or hydrolysed protein diet, although there are fewer options of

a hydrolysed diet with low-fat content, which frequently have 25-52 g fat/Mcal ME. Overall, it is still difficult to assess the dietary characteristics that will lead to the best outcome in these specific cases (Tolbert et al. 2022), but in the long-term, PLE dogs can be stable with these low-fat type diets (Hall and Day 2017). It appears that some Yorkshire Terriers will react to this diet for a long time without supplementary treatment (Rudinsky et al. 2017). Bota et al. (2016) also documented a general improvement (>50%) in Yorkshires diagnosed with PLE treated with a combined therapy of immunosuppressants, antibiotics and a low-fat hyperdigestible diet.

Fibre-enriched diets (>40g TDF/Mcal), or fibre supplementation (psyllium) are usually implemented in dogs presented with large intestinal clinical signs, especially used in chronic idiopathic colitis. These dogs might also benefit from a combination of a highly digestible diet alongside fibre supplementation (Leib 2000; Tolbert et al. 2022). Despite the decreased digestibility effect, fibre has some role in toxin-binding, water-holding, motility-regulation, and some prebiotic properties (Hall 2017).

Gluten-free diets are necessary in cases of gluten enteropathy, especially reported in Irish Setters and Border Terriers, an autosomal recessive familial condition (Rudinsky et al. 2018). Its success depends on excluding gluten from diet, ruling out wheat, barley, or rye (Hall and Day 2017). There are specific gluten-free commercial diets, and some hydrolysed dietary options also lack gluten (Kathrani 2020), offering more accessible options for veterinary practitioners.

1.4.3.2.3. Overview of studies that evaluated diet impact in CE

A retrospective study led by Makielski et al. (2019) summarised a few studies throughout some extensive literature regarding the impact of diet on canine CE. It showed strong evidence supporting the recommendation of an exclusion diet in dogs with CE. In addition, this study concluded that it was unfeasible to confirm which form of diet (novel protein source or hydrolysed diet) was most effective in improving GI signs. According to several studies, FRE represents 50-70% of CE cases in dogs, mainly in referral practices (Marks et al. 2002; Allenspach, Steiner et al. 2006; Luckschander et al. 2006; Allenspach, Wieland et al. 2007; Gaschen L et al. 2008; Schreiner et al. 2008; Mandigers et al. 2010; Volkmann et al. 2017).

For this project, a summary of various studies that evaluated the diet impact in dogs with GI diseases was conducted and is represented in Table 3.

Table 3-Overview of several studies concerning hydrolysed and hyperdigestible diets, novel protein commercial and homemade diets, low-fat and fibre-rich diets in GI diseases

Disease/ inclusion diagnosis	Study-reference	Dietary strategy/food type	Brand	Number of dogs*	Treatment sucess (%) †	Outcome	Treatment duration/ #time to achieve improvement	Duration of study	Medical evidence
CE	**Marks et al. 2002**	hydrolysed	Purina HA®	3 (6)	50%	Complete remission of clinical signs	10 w	10 w	IV
	Mandigers et al. 2010		RC HA®	11 (18)	61%	In terms of long-term clinical remission, hydrolysed diet was superior to a control diet (highly-digestible diet). 88% effective short-term	3y	36 months	II
	Walker et al. 2013		Purina Pro Plan HA®	20 (29)	69%	Remained asymptomatic or with trivial clinical signs	6w		III
	Allenspach et al. 2016	elimination or hydrolysed	NS-elimination diet (73 dogs) hydrolysed (58) and home-cooked (2)	131 (203)	65%	At 1y better outcome in FRE vs ARD and IRE. The type of diet did not have an effect on the outcome.	12w#	6-12 months	III
	Wang et et al 2019	hydrolysed	RC HA® dry dog	20 (29)	69%	Remission in 2w with a decreased CCEAI score from 4.1 to 1.3	2w#	42 d	III
	Luckschander et al. 2006	limited ingredient/novel protein source	Salmon and Rice diet	39 (65)	60%	Showed a decresead in CIBDAI score after treatment	10d		II or III
	Sauter et al. 2006		LA Salmon and Rice, Purina veteriary diets®	10 (21)	100%	Showed a decresead in CIBDAI score after treatment, the other 11 of them were on probiotic cocktail trial (1g/day)	4w		II
	Allenspach et al. 2007		LA Salmon and Rice, Purina veteriary diets®	39 (70)	56%	Elimination diet associated with long-term remissionnin dogs with FRE	3y	36 months	II
	Mandigers et al. 2010	higly-digestible	RC Intestinal diet®	1 (6)	17%	Less long-term remission compared to hydrolysed diet 88% effective in short-term	3y	36 months	II
Chronic colitis	**Simpson et al. 1994**		Pedigree Canine Selected Protein diet; Waltham® with protein sources limited to chicken and rice	10 (11)	90%	Within 1 month, 60% of the dogs required no sulphasalazine (or <) than when they were first exposed; 90% were stabilised with no medication treatment after two months.	after 2 months#	4 months	IV
PLE	**Simpson and Jergens 2011**	hydrolysed	NS	2 (6)	33%	N/A	N/A	N/A	V
	Rudinsky et al. 2017	elimination/ low fat/ hydrolysed	Diets included: Home-cooked (5), Gastrointestinal Low Fat, RC® (4), Hill 's i/d Low Fat® (1), Purina HA® (1)	8 (11)	73%	Significant improvements in serum albumin and CCECAI scores	1w-1month#	2-4w or 2-4months	IV
Chronic colitis	**Leib 2000**	fiber-specific or supplemeted	Soluble fiber (psyllium-Metamucil) wiith: Highly digestible diet -Hills i/d® (29) Hills k/d® (1), Hills d/d® (1), a combination of Hills d/d® and w/d® (1) or Purina EN® (1). Febendazole+ homemade diets, Hills i/d® or combination of Hills i/d and w/d® (3)	29 (36) includes the 3 dogs treated with febendazole	80%	Very good to excellent response to treatment with soluble fibre supplement in mostly highly digestible food cases (Hills i/d®).	3-34 months	3-34 months	IV

Legend: NS-non specified, N/A- not-available, *Number of dogs responding to diet trial, with total dogs in the study in brackets, † Percentage of dogs responding to diet alone in each study, #time to achieve improvement. CE-Chronic Enteropathy, PLE-Protein-losing Enteropathy, FRE- Food-responsive-Enteropathy, ARD-Antibiotic-responsive Diarrhoea, HA-Hypoallergenic, RC-Royal Canin, w-weeks, d-days. Evidence-based medicine levels: I-highly quality randomised study, II-lesser-quality randomised trial or prospective comparative study, III-case-control study or retrospective comparative study, IV-case series, V-expert opinion

1.4.3.3. Antimicrobials trial

Usually, most of ARD cases are younger dogs of large breeds, mainly German Shepherds (Dandrieux 2016). To consider an idiopathic ARD, first there should be a positive response to an AB trial; secondly, with a withdrawal of antibiotics (AB) there is usually a relapse of clinical signs; thirdly, remission when reintroduction of this treatment; and finally, elimination of other causes and assessment of histopathology (German et al. 2003; Hall and Day 2017).

Antimicrobial trials for a long time have been listed as an early diagnosis tool and as a second line empirical treatment (Dandrieux 2016). This approach is becoming more controversial as the incidence of ARD is low (Walker and McMahon 2019) and indiscriminate use of antibacterial drugs has severe individual and public health consequences (Cerquetella et al. 2020). The most frequent AB used for this purpose are tylosin (20 mg/kg PO q12h), metronidazole (10-15 mg/kg PO q12h), and oxytetracycline (10-20 mg/kg PO q8h), which are recommended for 4-6 weeks initially (Makielski et al. 2019). Less applied, sulfasalazine has an anti-inflammatory effect exclusively in the colon, 20-40 g/kg q8h for three weeks, followed by 20-40 mg/kg q12h (three weeks) and 10-20mg/kg q12h (three weeks) (Walker and McMahon 2019).

In dogs with CE, microbial dysbiosis is expected to have a perpetuating influence on mucosal inflammation (Allenspach and Mochel 2022). There are anecdotal reports of some antimicrobials' immunomodulatory and anti-inflammatory impact in CEs (Cerquetella et al. 2020), for instance, Shakir et al. 2011 verified this effect with metronidazole. Furthermore, Kilpinen et al. (2015) demonstrated a possible probiotic action in dogs with tylosin-responsive-diarrhoea.

Despite these benefits, it is recognised that dysbiosis also results from the use of these drugs (Suchodolski 2016; Cerquetella et al. 2020). In healthy dogs, both tylosin and metronidazole have been reported to significantly change the jejunal and colonic microbiota and metabolome (Suchodolski et al. 2009; Igarashi et al. 2014; Manchester et al. 2019). In addition, Jergens et al. (2010) showed that prednisone PO alone is as clinically effective as prednisone in combination with metronidazole PO in dogs with IBD. Other negative consequences of AB include the predisposition of patients to antimicrobial resistance (Walker and McMahon 2019), and the risk implicated considering dogs are potential reservoirs of some antibiotic-resistant strains that are probably hazardous to humans (Cerquetella et al. 2020).

Therefore, Cerquetella et al. (2020) argue that AB should not be used as a routine in the management of CD, they must be implemented only in specific conditions. Namely, after exclusion of other causes of CD that would not directly benefit from antibiotics; after diet trial and histopathology, or even after immunosuppressants in cases of unavailability for histopathology; and lastly, in cases of true primary infections with systemic signs or a known

pathogen non-self-limiting. Countless authors also believe there are alternative strategies to modulate the microbiota that should be advised considering antibiotics' negative effects, including probiotics, prebiotics, symbiotics and faecal microbiota transplant (FMT) (Cerquetella et al. 2020), despite their limited evidence on ARD cases (Walker and McMahon 2021).

1.4.3.4. Probiotics

In dogs with CE, dysbiosis has been observed, and probiotics and prebiotics have been suggested as strategies to promote a healthy faecal microbiota. Unfortunately, there isn't enough research to support their effectiveness in canine CE, hence they can not be scientifically advised (Walker and McMahon 2021).

The majority of studies available, in human and animal research, were performed with strains of VSL#3® probiotic but not with *Enterococcus faecium sf68*, which is used and sold routinely in clinics as Fortiflora®. Therefore, more investigations are needed regarding specific strains used in companion animals (Bybee et al. 2011; Lucena et al. 2018). Additionally, one study compared dogs exclusively on probiotics and another group with the conventional treatment (prednisone combined with metronidazole) and both groups had a significant improvement, though dogs with probiotics needed a longer period to achieve remission (Rossi et al. 2014). Recently, Jensen and Bjørnvad (2019) concluded that probiotic supplements did not add any significance to clinical improvement in chronic GI diseases, emphasising the role of diet as the main factor in achieving clinical improvement.

1.4.3.5. Immunosuppressive trial

Immunosuppression is one of the significant therapeutic points in managing of IBD. The latter disease seems to develop mainly in middle-aged dogs, with intermittent signs with an early beginning in age (Hall and Day 2017).

Many randomised control trials contribute to high-quality evidence for the recommendation of administration of glucocorticoids in dogs with IBD. Generally, prednisone or prednisolone is administered as a first immunosuppressant therapy in immunosuppressive doses (1-2 mg/kg PO q12h) (Makielski et al. 2019; Viviano 2022). If no response noticed or appearance of adverse reactions related to glucocorticoids, as well as frequent relapses when reducing the dosage, dogs may benefit from a second immunosuppressant drug (Viviano 2022). Azathioprine (2mg/kg PO q24h) is frequently used in association with glucocorticoids in dogs. It requires a 3-week period to achieve an effect, and careful monitoring is necessary due to its myelosuppressive action. Another option is chlorambucil (2-6mg/m^2 PO q24h), its combination with prednisolone has shown more significant results than azathioprine in the same association with prednisolone in RCE. Cyclosporine (5mg/kg PO q24h) can also be efficacious in combination with prednisolone, which is usually well accepted by dogs with CE

and PLE. It can be used as a last resource in cases non-responsive to prednisolone (Dandrieux 2016; Hall and Day 2017).

Furthermore, different individual trials have demonstrated that single-drug therapy with prednisolone is as effective as budesonide solely and in combination with metronidazole (Makielski et al. 2019).

1.4.3.6. Non-responsive to treatment and therapeutic alternatives

As no response to all empirical treatments is observed, dogs are diagnosed with refractory chronic enteropathy (RCE) (Rudinsky et al. 2017). These are difficult to identify and diagnose clearly (Benvenuti et al. 2021). The study conducted by Benvenuti et al. (2021) evidenced that 9-11% of dogs were non-responsive to empirical treatment. These dogs often have a decreased body condition, hypoalbuminaemia, and dilatation of chyliferous ducts. Although some RCE cases may not respond to the conventional treatment (diet, antibiotics, corticoids), they might react to other immunosuppressives (Masashi et al. 2012). Overall, cyclosporine and chlorambucil are the preferred choices in RCE cases (Dandrieux 2016), although they are also used in combination with prednisolone (Dandrieux, Noble et al. 2013; Hall and Day 2017).

Another new alternative therapy to consider is FMT. According to Igarashi et al. (2014) and Berlanda et al. (2021), RCE dogs treated with FMT had a complete GI clinical remission, and their microbiota was similar to healthy dogs. In general, the FMT principle is to restore or restructure the GI microbiota, which might resemble the dog's healthy gut and influence the intestinal immunological system. This is considered a last resource therapy in dogs because its safety is yet to be determined, and there is a risk of transmission of infectious agents (Walker and McMahon 2021).

1.5 Clinical scoring indices and monitorisation

The CIBDAI and CCECAI are clinical scoring indices broadly used and accepted to assess clinical improvement (Cerquetella et al. 2020) associated with the empirical treatments and to estimate clinical severity (Dandrieux 2016).

The CIBDAI evaluates six factors (0-3 each): attitude/ activity, appetite, vomiting, stool consistency, stool frequency, and weight loss. Based on the sum of each score, it classifies the disease as insignificant (0–3), mild (4–5), moderate (6–8), or severe (≥9) (Jergens et al. 2003). The CCECAI includes the parameters above and adds albumin concentration, presence of ascites, peripheral oedema, and pruritus. The latter classifies the disease as insignificant (0–3), mild (4–5), moderate (6–8), severe (9–11), and very severe (≥12) (Allenspach, Wieland et al. 2007). These clinical score systems have a subjective nature and clinical signs do not

directly relate to intestinal inflammation since there is no clear agreement of correlation with histopathology (Heilmann et al. 2018).

Section 3- THE IMPACT OF DIET IN THE MANAGEMENT OF CANINE CHRONIC DIARRHOEA: FROM CLASSIC TO REFRACTORY CASES

Part 1- The role of hydrolysed diet in the management of chronic diarrhoea in dogs-is it always the first therapeutic step?

1. Introduction and objectives

Canine CD is one of the most frequent complaints in veterinary practice (Walker and McMahon 2019). Exclusion of specific causes, such as infectious, mechanical, or extragastrointestinal diseases, must be performed with complete blood work, biochemistry, urinalysis, faecal exams, diagnostic imaging or other specific tests such as basal cortisol and pancreatic biomarkers (Hall and Day 2017). After this exclusion, sequential empirical treatments should be followed in order to achieve a definitive diagnosis and manage clinical signs (Walker and McMahon 2019).

Diet has a major role in the therapeutic line of canine CD, and it is usually the first approach after parasiticides (Dandrieux 2016). It has numerous benefits such as helping modulate the microbiota, immune system and motility, as well as the enhancement of the epithelial barrier function (Kathrani 2021). Generally, hydrolysed or novel protein diets are used with a good response and have a better long-term outcome (Dandrieux 2016; Makielski et al. 2019).

Despite the uncertainty of the best sequential treatment (Cerquetella et al. 2020), there is a significant consensus on the importance of a dietary trial in the management of canine CD as a first-line treatment (Tørnqvist-Johnsen et al. 2020). This trial should be performed prior to invasive diagnosis approaches, namely endoscopy and histopathology, as well as the implementation of antimicrobial and immunosuppressant drugs (Hall and Day 2017; Walker and McMahon 2019; Cerquetella et al. 2020). However, it is essential to understand whether these steps are followed before referral in the current clinical practice.

Therefore, sharing the same methodology and considering a sample of cases consulted for referral or second opinion of CD, this study aims to assess which percentage of canine cases is referred or consulted in second opinion and have never had a hydrolysed diet trial prior to these consultations, as well as to evaluate the relevance of this step in the therapy approach of CD cases.

2. Material and Methods

A retrospective multicentric study was conducted involving three referral centres: The HEV-FMV-University of Lisbon, in Portugal, The Oniris Ecole Nationale Vétérinaire Agroalimentaire et de l'Alimentation-Nantes, in France, and The Anicura Ospedale Veterinario-Portoni Rossi-Bologna, in Italy.

2.1. Sample population

Medical records of dogs consulted in the three referral centres, from April 2018 to December 2021, were reviewed. Dogs of all sexes, breeds, and ages presenting CD (>three weeks duration), in a referral or second opinion consultation by an ECVIM-diplomate or a resident (under supervision) were included.

To be included in the study, dogs must have had a complete medical and dietary history, as well as a follow-up of at least one month. This follow-up was based on the database's medical records and email contacts. All data regarding deworming; supportive therapies, such as antiemetics (AEs), probiotics (PBs), gastric protectants (GPs), cobalamin supplementation, fibre source (FS), faecal microbiota transplant (FMT), lactulose; antibiotics and immunosuppressants were assessed.

Dogs were excluded if there was a confirmed infectious disease diagnosis, including bacterial, parasitic (*Giardia*) or viral agent; confirmed or suspected extragastrointestinal causes of CD; GI tumours, and mechanical aetiologies (ileus, obstructions, or intussusception). In addition, cases with concomitant metabolic, endocrine diseases (e.g., diabetes mellitus, renal disease) or other illnesses under another specific type of diet directed to those conditions were excluded.

2.2. Study Group 1 (SG1)

From the sample population selected, in line with inclusion and exclusion criteria already mentioned, dogs were evaluated depending on whether they had been fed a hydrolysed diet prior to referral. If dogs had never tried these diets before, they were allocated to the Study Group 1 (SG1).

Data concerning the first consultation as a referral, or second opinion was detailed. As a first step, severity of clinical signs was retrospectively assessed according to the CIBDAI score (Jergens et al. 2003). Clinical signs were classified as insignificant (0–3), mild (4–5), moderate (6–8), or severe (≥9) (Jergens et al. 2003). Thereafter, cases were assessed according to concurrent treatment when consulted. For this purpose, dogs were categorised in line with their main treatments, more specifically if they were not under any drug treatment, or if they were being treated with supportive therapies (AEs, PBs, GPs, cobalamin, FS, FMT or lactulose), with or without antibiotics or immunosuppressants.

At referral or second opinion consultation, a hydrolysed diet trial was the first treatment approach in SG1 cases. Depending on concurrent medical treatment readjustments, dogs were allocated either to Group A or B. When diet was the only therapeutic decision, and the former ongoing treatments were maintained, dogs were assigned to Group A. If apart from diet, other ancillary treatments were concurrently prescribed, cases were allocated into Group B.

In Group A, if dogs had ongoing therapies, these were appropriately distinguished as supportive, antibiotics or immunosuppressants treatments. For Group B, the main treatments considered were: supportive treatments (AEs, GPs, PBs, FS, cobalamin supplementation, FMT, FS or lactulose) ± antibiotics ± immunosuppressant therapy. These treatments were described in more detail in line with their category

Clinical improvement of all SG1 cases, including Group A and B, was assessed with a follow-up of at least one month after the diet transition. This was also evaluated separately, in line with the main treatment categories in Group A and B.

In Group A, and Group B's main categories (supportive, antibiotics and immunosuppressants), dogs were characterised according to initial disease severity by CIBDAI scores calculated retrospectively, in agreement with clinical signs reports when first consulted in the referral or second opinion consultations, correlating these findings with clinical improvement. Furthermore, the clinical improvement of these dogs was related to their initial CIBDAI scores. Figure 1 illustrates the study scheme.

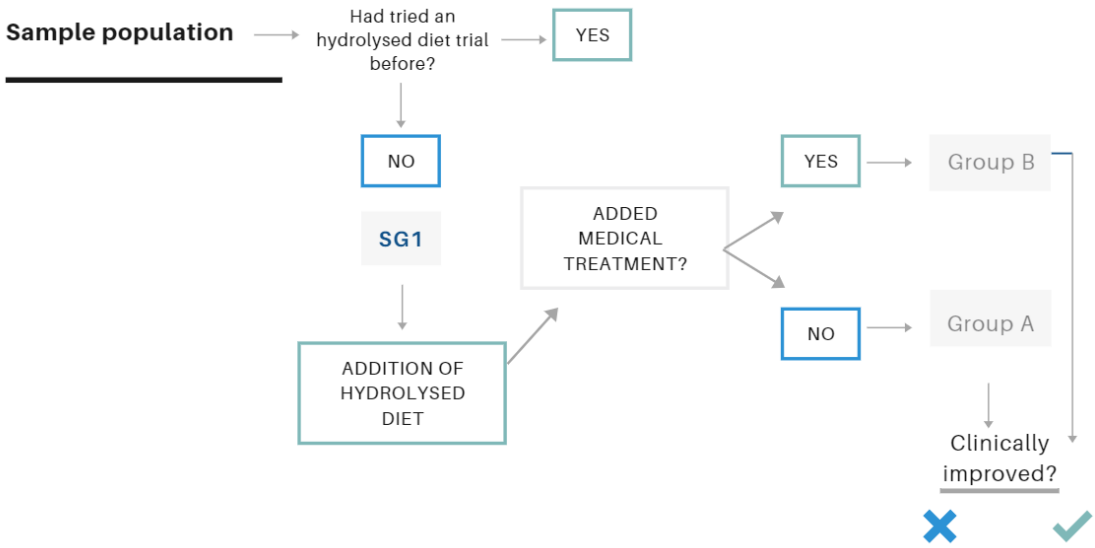


Figure 1- Scheme of SG1 selection and analysis

2.3. Data processing and statistical analysis

All collected data from medical records were recorded and a database was created. Descriptive statistical analysis was performed using Microsoft® Excel® 2022. Results are reported as absolute numbers and percentages (%).

In order to compare the disease's severity (insignificant, mild, moderate or severe) using total CIBDAI scores of the improved and unimproved cases of SG1 within the different groups A and B, a Fisher's exact test was applied. The commercial statistical programme IBM® SPSS® Statistics version 28 was used to implement Fisher's exact tests. The significance threshold was set at a p-value of 0.05 for a confidence interval of 95%.

3. Results

3.1. Sample population Characterisation

From medical records between April 2018 and December 2021, a total of 142 dogs presented themselves with CD as a referral or second opinion consultations and were included in the study. In detail, 78 cases were from HEV-FMV-University of Lisbon, 10 from Oniris Ecole Nationale Vétérinaire Agroalimentaire et de l'Alimentation, and 54 dogs from Anicura Ospedale Veterinario-Potoni Rossi-Bologna, Italy.

Information about gender, age and breed was collected. A total of 64% were males (91/142), ranging from 5 months to 15 years old. Females accounted for the remaining 36% (51/142) of the sample (Table 4), presenting ages ranging from 6 months to 16 years old. Concerning age, a median of 5 years old (Interquartile Range (IQR)=5) was present in the total sample population.

Table 4- Gender characterisation of dogs presenting for chronic diarrhoea

SEX	n	(%)
FEMALE	51	35,92 %
MALE	91	64,08 %
TOTAL	142	100 %

A total of 18.3% (26/142) of dogs were of mixed breed. German Shepherds, French Bulldogs, and Labrador Retrievers were overrepresented when compared to the other breeds (7-11%). Results are summarised in Table 5.

Table 5- Breed characterisation of sample population

Breed	*n*	*(%)*	*Breed*	*n*	*(%)*
Mixed Breed/Undefined	26	18,3%	American Bully	1	0,7%
German Shepherd	15	10,6%	Argentine Dogo	1	0,7%
French Bull Dog	13	9,2%	Bernese Mountain dog	1	0,7%
Labrador Retriever	10	7,0%	Bull Terrier	1	0,7%
Yorkshire Terrier	8	5,6%	Cavalier King Charles Spaniel	1	0,7%
Jack Russel Terrier	6	4,2%	Coton de Tuléar	1	0,7%
Chihuahua	5	3,5%	English Bulldog	1	0,7%
Golden Retriever	5	3,5%	English Setter	1	0,7%
Maltese Bichon	4	2,8%	Epagneul Breton	1	0,7%
Podengo	4	2,8%	Great Dane	1	0,7%
American Stafford Terrier	3	2,1%	Irish Setter	1	0,7%
Pinscher	3	2,1%	Japonese Spitz	1	0,7%
Akita Inu	2	1,4%	Kurzhaar	1	0,7%
Boston Terrier	2	1,4%	Pitbull	1	0,7%
Boxer	2	1,4%	Poodle	1	0,7%
English Cocker Sapniel	2	1,4%	Pug	1	0,7%
Lagotto Romagnolo	2	1,4%	Rottweiler	1	0,7%
Pomeranian Lulu	2	1,4%	Samoieda	1	0,7%
Portuguese Water Dog	2	1,4%	Shih-tzu	1	0,7%
Weimaraner	2	1,4%	Siberian Husky	1	0,7%
West Highland White Terrier	2	1,4%	Spanish Mastiff	1	0,7%
			Star Mountain	1	0,7%

Legend: n of sample population =142 dogs

3.2. SG1 Characterisation

Of the 142 dogs included in this study, 81 cases (57%) had never been fed a hydrolysed diet prior to these consultations (Graphic 2) and were included in SG1. The age presented a median of 6 years old (IQR=5,25), being 65% (53/81) male and 35% (28/81) female dogs. A total of 22.2% (18/81) were of mixed breeds, followed by Labrador Retriever (11,1%-9/81) and German Shepherd (9,9%-8/81). The remaining breeds are characterised in Table 6.

Graphic 2- Proportion of sample population and SG1

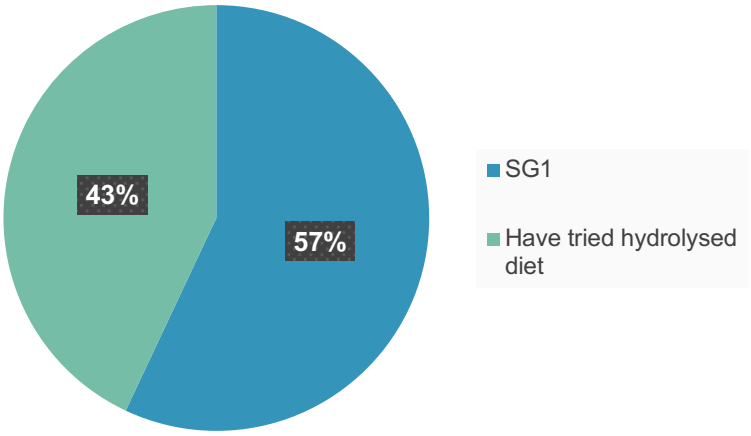


Table 6- Breed characterisation of SG1

Breed	*n*	*%*
Mixed Breed/Undefined	18	22,2%
Labrador Retriever	9	11,1%
German Shepherd	8	9,9%
Jack Russel Terrier	5	6,2%
French Bulldog	5	6,2%
Podengo	3	3,7%
Yorkshire Terrier	3	3,7%
Chihuahua	3	3,7%
Golden Retriever	2	2,5%
Boston Terrier	2	2,5%
Portuguese Water Dog	2	2,5%
Lagotto Romagnolo	2	2,5%
Maltese	2	2,5%
WHWT	1	1,2%
Poodle	1	1,2%
American Bully	1	1,2%
Japonese Spitz	1	1,2%
Bull Terrier	1	1,2%
English Bulldog	1	1,2%
Epagneul Breton	1	1,2%
Cavalier King Charles Spaniel	1	1,2%
Argentine Dogo	1	1,2%
Akita Inu	1	1,2%
Pinscher	1	1,2%
English Cocker Spaniel	1	1,2%
Bernese mountain dog	1	1,2%
Boxer	1	1,2%
Rottweiler	1	1,2%
Coton de Tulear	1	1,2%
Kurzhaar	1	1,2%

Legend: n of SG1 =81 dogs

3.3. SG1 Analysis

3.3.1. Initial CIBDAI scores

Of the 81 cases (SG1), 38,3% (31/81) had a moderate disease severity, followed by 32,1% (26/81) and 21% (17/81) with mild and insignificant disease, respectively. Only 8,6% (7/81) were classified with severe illness. These classifications are described in Table 7.

Table 7- Classification of SG1 initial disease's severity with total CIBDAI scores

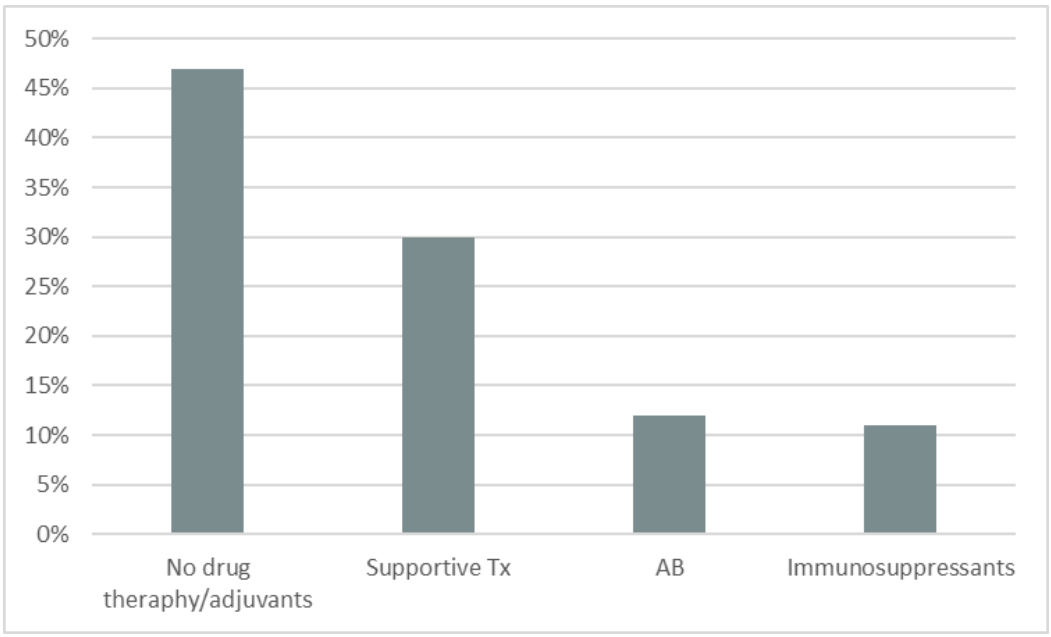
TOTAL CIBDAI SCORE-SG1 CLASSIFICATION OF INITIAL DISEASE SEVERITY	n	%
INSIGNIFICANT (0–3)	17	21%
MILD (4–5)	26	32,1%
MODERATE (6–8)	31	38,3%
SEVERE (≥9)	7	8,6%

Legend: n of SG1 =81 dogs

3.3.2. Treatments at presentation

When consulted, a total of 47% (38/81) from SG1 were not under any medication. A total of 30% (24/81) were on supportive therapy, 12% (10/81) were receiving antibiotics, and 11% (9/81) were already on immunosuppressants at the time (Graphic 3).

Graphic 3- Proportion of dogs from SG1 according to medication at presentation



Concerning the 24 dogs on supportive therapy, 50% (12/24) of them were on PBs (Table 8). In Table 8, the ongoing supportive therapies are described according to GPs, AEs, PBs, cobalamin and others previously implemented. In addition, the details of each drug or ancillary therapies used are explicit in Table 9.

Table 8- Percentage of supportive treatments used in each treatment category (GPs, AEs, PBs, Cobalamin and others) at presentation

	SUPPORTIVE TX (n=24)				
	GPs	**AEs**	**PBs**	**Cobalamin**	**Others**
n	8	3	12	2	2
%	33,3%	12,5%	12,5%	8,3%	8,3%

Legend: Tx-Treatment, GPs-Gastric protectants, AEs-Antiemetics, PBs-Probiotics.

Table 9- Drugs used in each supportive treatment category (GPs, AEs, PBs and others) at presentation

GPs (n=8)	Sucralfate (n=1)	Omeprazole (n=4)	Pantoprazole (n=2)	Ranitidine (n=1)		
AEs (n=3)	Maropitant (n=3)					
PBs (n=12)	Fortiflora® (n=4)	VSL#3 ® (n=2)	Vivomixx ® (n=2)	Enterogermina (n=1)	Enterofilus (n=1)	Florentelo (n=2)
Others (n=2)	Psyllium (n=1)	Lactulose (n=1)				

Legend: GPs-Gastric protectants, AEs-Antiemetics, PBs-Probiotics.

Antibiotics were being administered to 10 dogs both in the past and during consultation. Detailed antibiotics' drug information is specified in Table 10. Metronidazole was ongoing in 60% (6/10) of these dogs. Additionally, some of these 10 dogs had other ongoing ancillary treatments applied simultaneously, including 3 dogs on PBs (2 with Vivomixx® and the other no information was available), 4 on GPs (one with omeprazole, another with esomeprazole and the other 2 with ranitidine), and lastly 2 patients were on cobalamin supplementation.

Table 10- Drug discrimination of antibiotics already ongoing at presentation

	ANTIBIOTICS (n=10)					
	Metronidazole	**Cephalexin**	**Cefovecin**	**Enrofloxacin**	**Amoxicilin/ clavulanic acid**	**Spiramycin**
n	6	1	1	1	1	2*
%	60%	10%	10%	10%	10%	20%

Legend: *in combination with metronidazole

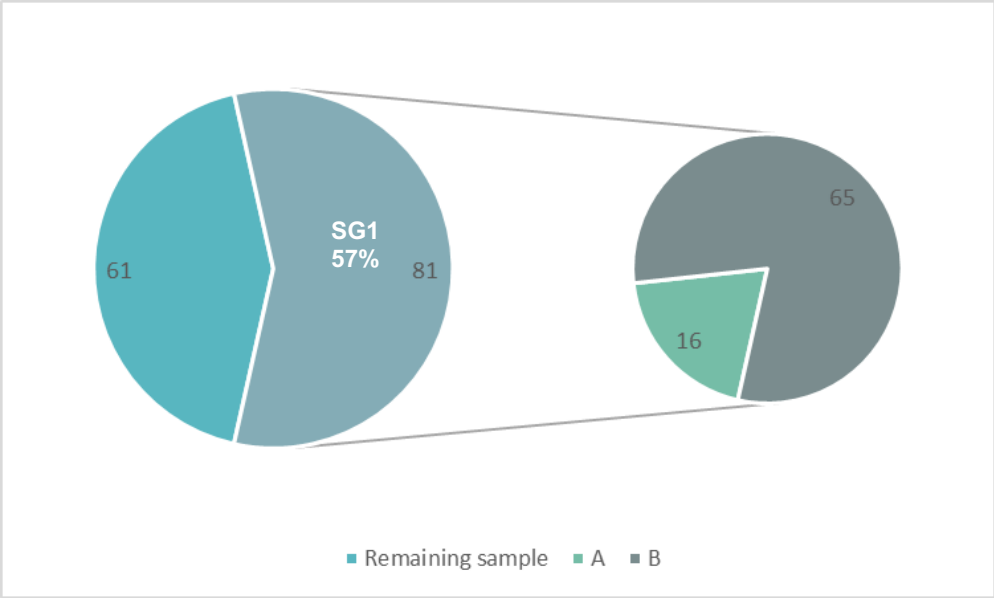
Regarding the dogs on immunosuppressants (n=9), 8 were on prednisolone, and 1 was treated with a combination of prednisolone and cyclosporine. Barring these medications, 2 were on antibiotics (metronidazole), 1 with AEs (maropitant), 5 with GPs (omeprazole), and another one with PBs (Fortiflora®).

3.3.3. Diet implementation and other ancillary therapies

All the 81 dogs included in SG1 started a hydrolysed diet trial at consultation. In detail, 82,72% (67/81) of dogs were introduced to Purina ProPlan Hypoallergenic® (HA), 7,41% (6/81) were fed with Royal Canin (RC) Hypoallergenic®, 6,17% (5/81) with Affinity Advanced Hypoallergenic® and 3,70% (3/81) with Hills z/d®.

This diet transition was the only treatment decision in 20% of dogs (16/81), forming Group A. In the remaining 80% (65/81) cases, this new hydrolysed diet was prescribed in association with other main treatments, composing Group B (Graphic 4).

Graphic 4- Number of dogs composing SG1, Group A and B



3.3.3.1. Group A

Cases from Group A (n=16) did not add any further treatment additionally to the hydrolysed diet. Although 4 dogs were without any medication, and in the remaining 12 cases current treatments were maintained, detailing: 4 on supportive treatment, 3 on antibiotics and 5 on immunosuppressants.

3.3.3.2. Group B

In Group B (n=65), 62% (40/65) added or changed supportive therapy, 18% (12/65) started antibiotics and 20% (13/65) immunosuppressants.

Of the 40 dogs on supportive treatments, 70% (27/40) added PBs alongside the hydrolysed diet implementation (Table 11). Table 11 summarises the supportive therapies added in their respective categories. Moreover, Table 12 specifies in detail the type of supportive treatments added within GPs, AEs, PBs, B12 and others.

Table 11- Percentage of supportive treatments in Group B (GPs, AEs, PBs, Cobalamin and others)

	SUPPORTIVE TX (n=40)				
	GPs	**AEs**	**PBs**	**Cobalamin**	**Others**
n	8	10	27	17	6
%	20%	25%	70%	43%	15%

Legend: Tx-Treatment, GPs-Gastric protectants, AEs-Antiemetics, PBs-Probiotics

Table 12- Discrimination of supportive treatment drugs added of each category (GPs, AEs, GPs, PBs, others) in Group B

GPs (n=8)	Sucralfate (n=1)	Omeprazole (n=6)	Esomeprazole (n=1)	
AEs (n=10)	Maropitant (n=10)			
PBs (n=27)	Vivomixx® (n=22)	VSL#3 ® (n=3)	Fortiflora ® (n=2)	Florentelo ® (n=1)
Others (n=6)	Psyllium (n=1)			

Legend: GPs-Gastric protectants, AEs-Antiemetics, PBs-Probiotics

Furthermore, from dogs to which antibiotics were added besides dietary management (n=12), 83,33% (10/12) patients received, metronidazole, one dog was introduced to enrofloxacin, another doxycycline, and lastly another dog added sulfasalazine combined with metronidazole. Nevertheless, PBs were included in 25 % (3/12) of these dogs (2 with *Vivomixx*® and one with Fortiflora®), AEs were prescribed to one dog (maropitant) and GPs to another patient (omeprazole).

Finally, from the cases that started immunosuppressants (n=13), prednisolone was added in 92,3% (12/13) and chlorambucil was introduced to one dog (0,7%-1/13). Apart from the immunosuppressants, in these 13 dogs, antibiotics were implemented in 3 cases (2 with metronidazole and one with enrofloxacin), AEs in 3 dogs (all with maropitant), GPs in 6 dogs

(4 with omeprazole, one with sucralfate and another with a combination of both), and lastly cobalamin supplementation was needed in another dog.

3.3.4. Clinical improvement and relationship with CIBDAI initial scores

A general clinical improvement was documented in 70,4% (57/81) of SG1 (n=81). These cases are represented by 50% (8/16) of dogs from Group A (n=16), in which a hydrolysed diet was the only novel therapeutic approach, and 75% (49/65) cases from Group B (n=65).

Overall, 29,6% (24/81) of dogs from SG1 had no response documented, detailing 50 % (8/16) from group A and 25% (16/65) from Group B.

3.3.4.1. Group A

Within the 16 dogs from Group A, 50% (8/16) had a visible clinical improvement. Of the patients that did not have any ongoing therapies (n=4), 75% (3/4) improved with the new diet. Within the cases that maintained supportive treatments (n=4), 50% (2/4) had a positive response, as well as 66,7% (2/3) of dogs with antibiotics (n=3). Lastly, of the dogs already on immunosuppressants (n=5), 40% (2/5) improved with the hydrolysed diet implemented.

Regarding Group A's initial CIBDAI scores, in the cases that improved (n=8), 75% (6/8) dogs were classified with an insignificant to moderate disease, and no dog was categorised with severe illness. In contrast, from cases that did not improve (n=8), 50% (4/8) had moderate disease and no dog was classified with an insignificant severity prior to the diet transition. Table 13 summarises these results.

Table 13- Initial CIBDAI classification in Group A according to clinical improvement after diet transition

	TOTAL CIBDAI INITIAL SCORE-SG1-GROUP A (n=16 DOGS)			
	Improved (n=8)		*No improvement (n=8)*	
	n	%	n	%
INSIGNIFICANT (0–3)	3	37,5%	0	0,0%
MILD (4–5)	3	37,5%	3	37,5%
MODERATE (6–8)	2	25,0%	4	50,0%
SEVERE (≥9)	0	0,0%	1	12,5%

A statistical association was not found when comparing the clinical improvement with the initial CIBDAI score classification applying Fisher's exact test (p=0,324) in this group.

3.3.4.2. Group B

Within the cases that changed or added supportive treatment (n=40), 80% (32/40) of them improved, whereas the other 20% (8/40) had no noticeable clinical improvement. From the former group, there was no case with severe disease, and from the latter group, 62,5% (5/8) had a moderate GI disease before the diet and addition of supportive treatment. Table 14 describes these results.

A statistical association was not found when comparing the clinical improvement with the initial CIBDAI score classification using Fisher's exact test (p=0,174).

Table 14- Initial CIBDAI classification in Group B-Diet combined with supportive therapy, according to clinical improvement after diet transition

	TOTAL CIBDAI SCORE-SG1-GROUP B-DIET+SUPPORTIVE TX (n=40)			
	Improved (n=32)		*No improvement (n=8)*	
	n	%	n	%
INSIGNIFICANT (0–3)	8	25,0%	1	12,5%
MILD (4–5)	11	34,4%	1	12,5%
MODERATE (6–8)	13	40,6%	5	62,5%
SEVERE (≥9)	0	0,0%	1	12,5%

Legend: Tx-Treatment

Regarding the group in which the addition of antibiotics was made concurrently to the new diet (n=12), 75% (9/12) improved with this combination, while 25% (3/12) did not show a positive response. From the improved dogs (n=9), 44,4% (4/9) cases were previously classified with a mild disease while from the unimproved group (n=3), 66,7% (2/3) dogs were categorised with an insignificant disease. Table 15 summarises these results.

A statistical association was not found when comparing the clinical improvement with the initial CIBDAI score classification using Fisher's exact test (p=0,523).

Table 15- Initial CIBDAI classification in Group B-Diet combined with antibiotic therapy, according to clinical improvement after diet transition.

	TOTAL CIBDAI SCORE-SG1-GROUP B-DIET+AB TX (n=12)			
	Improved (n=9)		*No improvement (n=3)*	
	n	%	n	%
INSIGNIFICANT (0–3)	1	11,1%	2	66,7%
MILD (4–5)	4	44,4%	1	33,3%
MODERATE (6–8)	3	33,3%	0	0,0%
SEVERE (≥9)	1	11,1%	0	0,0%

Legend: AB-Antibiotics, TX-Treatment

From the dogs that were started on immunosuppressants alongside a hydrolysed diet (n=13), 61,5% (8/13) of them improved clinical signs and 38,5% (5/13) of them had no improvement. Within the improved dogs (n=8), 37,5% (3/8) were classified according to initial CIBDAI with a severe disease. From the unimproved dogs (n=5), 60% (3/5) on this therapy combination, were classified on their first consultation with a moderate disease according to initial CIBDAI scores, and in this group, an insignificant disease was not classified in any of these dogs prior to these medical changes (Table 16).

A statistical association was not found when comparing the clinical improvement with the initial CIBDAI score classification applying Fisher's exact test (p=0,450).

Table 16- Initial CIBDAI classification in Group B-Diet combined with immunosuppressant therapy, according to clinical improvement after diet transition

	TOTAL CIBDAI SCORE-SG1-GROUP B-DIET+IMMUNOSUPPRESSANTS TX (n=13)			
	Improved (n=8)		*No improvement (n=5)*	
	n	%	n	%
INSIGNIFICANT (0–3)	2	25,0%	0	0,0%
MILD (4–5)	2	25,0%	1	20,0%
MODERATE (6–8)	1	12,5%	3	60,0%
SEVERE (≥9)	3	37,5%	1	20,0%

Legend: Tx-Treatment

4. Discussion

This first study contributed to assess the impact of hydrolysed dietary trials as a first-line treatment among canine CD cases in referral practice.

Most dogs from the sample population and SG1 were males, (64% and 65%, respectively). This finding is in conformity with the study conducted by Volkmann et al. (2017), in which males were overrepresented, and is also in line with other former reports (Craven et al. 2004; Munster et al. 2006; Allenspach, Wieland et al. 2007; Allenspach, Culverwell et al. 2016; Marchesi et al. 2017). Sex predisposition has not been described in dogs with GI disease, although there has been an overrepresentation of intact males, followed by spayed females (Couto et al. 1989; Craven et al. 2004; Allenspach, Steiner et al. 2006; Burgener et al. 2008; Mancho et al. 2011; Heilmann et al. 2014). Importantly, there was no formal comparison made of each referral centre during the same period, hence the importance of this finding is unknown.

In addition, the sample population was mainly composed of middle-aged dogs. This is similar to the Volkmann et al. (2017) and Benvenuti et al. (2021) studies. Also, most dogs were of mixed breeds, although German Shepherds, French Bulldogs and Labrador Retrievers had a higher prevalence compared to the other breeds. Indeed, German Shepherds have a predisposition to a variety of CD causes, including ARD, FRE, IBD and EPI (Walker and McMahon 2019). Additionally, this finding corresponds with the study led by Benvenuti et al. (2021), in which 22% (36/165) dogs were of mixed breed, followed by 16% (26/165) cases were German Shepherd dogs. Also, French Bulldogs are predisposed to GC (Hall and Day 2017) and Labrador Retrievers to FRE primarily (Walker and McMahon 2019), highlighting the prevalence of GI disease in both breeds.

Interestingly, more than half (57%-81/142) of the sample population had never tried a hydrolysed diet prior to these referrals or second opinion consultations. Indeed, it has been proven that both hydrolysed and non-hydrolysed diets can be successful in the management of GI diseases, at least for the short-term period. However, from a long-term perspective, hydrolysed diets have a better outcome (Dandrieux, Martinez et al. 2019), therefore an effort of trying hydrolysed diets should be made in order to assess clinical improvement. In addition, diet response is individualised and depends on the diet composition and content which differs dramatically in commercial diets, especially regarding protein ingredients and fat quantity (Tobert et al. 2022). Also, FRE is eventually detected in the majority of dogs who are referred for CE, as documented by Volkman et al. (2017) in which 66% of CE cases with CD were diagnosed with FRE. Alongside the reasons mentioned above, it is indeed advised that multiple diet trials are attempted before diagnosing a case as non-responsive food enteropathy (Tolbert et al. 2022). This also highlights the need of undertaking a proper diet trial before referrals,

preventing unnecessarily aggressive treatments, or allowing to lower antibiotic and immunosuppressant dosages (Isidori et al. 2022). Hence, a hydrolysed diet should have been implemented as one of the first therapies approaches in general practices as well.

At presentation, most dogs from SG1 (38,3%) were classified with moderate disease severity and a few with severe illness (8,6%). Both scores can have been justified by treatments already ongoing as it could have reduced the severity of clinical signs at presentation, or the GI disease was beginning to deteriorate. Although Tørnqvist-Johnsen et al. (2020) argued that CIBDAI can objectively assess the disease's severity in line with clinical signs, many still believe this is a subjective indicator (Im Hof et al. 2012; Grellet et al. 2013). In addition, there is no documented correlation between the severity of clinical signs and the magnitude of histologic lesions (Heilmann et al. 2014; Heilmann et al. 2018). As a result, attention should be given to the utility of this classification considering it does not indicate intestinal inflammation (Collins 2013), and it might not dictate the necessary treatment.

Nearly half (47%-38/81) of the SG1 dogs were not on medications or other ancillary treatments at the time of consultation. However, it is not known whether these dogs were previously under other type of diet or if medications were stopped shortly before consultation, which could have influenced the further outcome when diet was added to these dogs.

In the cases under supportive therapy, half of them (12/24-50%) were on PBs. Indeed, PBs have been considered one plausible and increasing alternative to antimicrobial trials (Cerquetella et al. 2020). According to several studies (Rossi et al. 2014; White et al. 2017; Rossi et al. 2018), PBs appear to be effective in cases of diarrhoea, even in dogs with IBD. They work primarily as an antagonist against undesired microbial species and enhance modulation of the immune intestinal system. In opposition to these facts, data from PBs studies is controversial (Isidori et al. 2022). As a matter of fact, in a recent review led by Jensen and Bjørnvad (2019), the authors stated that in GI chronic disease, PBs had no additional effect on the treatment with diet, emphasising the dietary impact as the main variable in the clinical outcome of these dogs.

About 12% (10/81) dogs were already on antibiotics without having experienced a hydrolysed dietary trial prior to these consultations. No data was collected on previous medical history, therefore, it was not possible to ascertain whether the use of these antibiotics was necessary. According to recent research (Cerquetella et al. 2020), antimicrobials should be limited to canine chronic diarrhoeic individuals with signs of true primary infection or following proper dietary trials and intestinal biopsies. In addition, most cases were receiving metronidazole (60%-6/10), one of the most frequently prescribed antibiotics for GI disease owing to its immunomodulatory effects (Dandrieux 2016). Another dog was treated with enrofloxacin, possibly due to a suspected GC, considering it is the first antibiotic choice for these cases (Walker

and McMahon 2021), however, in this particular case, there is no report of a diagnosis or confirmed GC. Also, there was one dog in which cephalexin was prescribed, one dog received cefovecin, another amoxicillin/clavulanic acid and two metronidazole combined with spiramycin, which are not the preferable choices in an antibiotic empirical treatment in CD cases (Dandrieux 2016; Westermarck 2016). Except for metronidazole and enrofloxacin, the remaining antibiotics already ongoing might have been implemented due to a possible concomitant disease. However, there are no evidence of these reasons on the dog's medical records.

Surprisingly, 11% (9/81) dogs were already on immunosuppressants without ever having tried a hydrolysed diet trial. In certain circumstances, it is true that corticosteroids and other immunosuppressants are needed to manage clinical signs, although they are usually prescribed only after other therapeutic options have failed, which is not observed in these cases as dietary trial was not correctly attempted (Cerquetella et al. 2020). Most of these dogs were on prednisolone and another patient was receiving a combination of prednisolone and cyclosporine. These findings are in agreement with the mainly used immunosuppressants in cases of suspected IBD (Makielski et al. 2019), when refractory to diet and following histopathology confirmation (Cerquetella et al. 2020). However, these cases did not undergo a proper dietary trial as hydrolysed diets were not experiment before aggressive therapy. Therefore, they could have been responsive to diet at the beginning of therapy using a hydrolysed dietary option.

In dogs that had never experienced a hydrolysed diet before (SG1), all the clinicians from a referral and second opinion consultations implemented it, as a first approach, even if some were already on antibiotics or immunosuppressants. Indeed, there is a strong consensus that the empirical treatment trials start with a feeding trial, especially using an exclusion diet, in the treatment of CD (Westermarck 2016; Tørnqvist-Johnsen et al. 2020). This first dietary decision in a referral setting, highlights that there is a strong belief on an appropriate hydrolysed dietary trial as a first-line therapy in cases of CD. However, in a general practice context, this scientific consensus was not followed prior to these consultations.

As previously mentioned, either a hydrolysed or a non-hydrolysed exclusion diet can have a successful effect for a short period in dogs with CE (Dandrieux, Martinez et al. 2019). However, the main reason for this primary choice has been justified by the better outcome in the long term with hydrolysed diets (Mandigers et al. 2010). Also, several studies using hydrolysed diets have demonstrated its effectiveness, as well as a high frequency of FRE cases diagnosed, which are represented between 50-70% of dogs, mainly in a referral context (Marks et al. 2002; Walker et al. 2013; Allenspach, Culverwell et al. 2016; Wang et al. 2019). Moreover, hydrolysed diets have the advantage of aiding the immune system, through the decreasing of protein diet antigens, the possibility of low-fat content which can be beneficial in suspected PLE cases, and the presence of omega-3 fatty acids and soy that have an immunomodulatory

effect (Kathrani 2021). Therefore, the various benefits associated with this specific diet support the use of a hydrolysed diet in these dogs.

Purina HA® was the preferable hydrolysed diet choice in SG1 as 82,72% (67/81) transitioned onto this diet, followed by other plausible hydrolysed diets available on the market (RC Hypoallergenic®, Affinity Advanced Hypoallergenic® and Hills z/d®). Besides the hydrolyse protein benefits, Purina HA® has in its composition 21% of protein, mainly from soy (Purina n.d.), which is known for its immunomodulatory effects (Kathrani 2021). This source of protein is less likely to be present in most common commercial diets and, as a result, it is expected that dogs have never been in contact with it resulting in a decreased chance of adverse food reactions (Cave 2006). Another component of Purina HA® is omega 3 fatty-acids (Purina n.d.), which have an identical immunomodulatory influence as soy (Kathrani 2021).This dietary choice (Purina HA®) might also have been grounded on clinician preference, patient dietary history and dog preference (Volkmann et al. 2017).

In these referral centres it was conceivable that a small percentage (20%-16/81) of dogs did not require therapy alterations besides diet (Group A). On the other hand, in a myriad of SG1 cases (80%-65/81) an addition or treatment changes were needed (Group B). Indeed, it is complicated to deny the dog owner's the use of drug therapies when severe clinical signs are present or are starting to surface (Westermarck 2016). In fact, in the study led by Tørnqvist-Johnsen et al. (2020), several owners did not accept monotherapy.

Overall, there were various treatment combinations present, hence, to simplify the understanding and impact of this study, a division of main treatments was established according to the empirical therapies known among the scientific community.

From Group B, 62% (40/65) dogs added supportive therapy alongside the new diet. Certainly, GI diseases might involve other clinical signs, such as vomiting present in cases of gastroenteritis in which AEs and GPs might be essential to assist these patients (Hall and Day 2017). In the majority of the dogs on supportive treatments (70%-28/40), PBs were employed. This could have been justified by the well-known PBs' benefits, although no scientific consensus supports the implementation of this supplementary treatment in CD due to the lack of evidence (Isidori et al. 2022). Vivomixx® was added to 22 dogs, whereas only 3 patients had VSL#3® prescribed. Indeed, several studies have shown VSL#3® efficacy in animals (Rossi et al. 2014; Jensen and Bjørnvad 2019). Notably, there is only one recent study describing the effect of Vivomixx® in dogs with IBD, in which the probiotic effect was compared to the combination of prednisone and diet. In fact, the tight junction protein expression was enhanced in the PBs' group, indicating that Vivomixx® may have a positive effect on mucosal homeostasis (White et al. 2017). Equally important, 43% (17/40) dogs required cobalamin supplementation, as the majority of these dogs had a cobalamin value <400 ng/L, which is in line with the Texas

A&M University Gastrointestinal Laboratory (2018) recommendations. Finally, in 6 dogs a FS (psyllium) was implemented in addition to diet. Indeed, these dogs had reports of large bowel clinical signs at presentation (haematochezia and mucous) which could have supported the use of this supplement as it has shown positive improvements in cases of chronic colitis (Isidori et al. 2022; Alves et al. 2021).

A relatively small proportion of dogs in Group B (18%-12/65), started an antibiotic trial, in which metronidazole was the preferred choice. This selection is consistent with the literature, as it is one of the first antibiotics used as empirical therapy for its immunomodulatory effect and impact on microbiota (Cerquetella et al. 2020). In one of the dogs, enrofloxacin was prescribed despite the lack of information regarding a definitive diagnosis of *Escherichia coli*. which is not in line with the literature's advise (Hall 2017; Walker and McMahon 2021). Doxycycline was implemented in another dog, an antibiotic not frequently used as empirical therapy in CD cases (Dandrieux 2016; Westermarck 2016). Nonetheless, the presence of a concomitant disease should be contemplated as justification for antibiotic use, although on the database this is not reported. Notably, antibiotics were prescribed only in a minor percentage of dogs, which can suggest that veterinarians are more aware of their negative effects, including future resistance to antibiotics and disturbance of the GI microbiota (Cerquetella et al. 2020).

Concerning dogs that added immunosuppressants, most of them (92,3%-12/13) were treated with prednisolone while one case was switched to chlorambucil. This is expected since glucocorticoids are mainly prescribed as first-line immunosuppressants while others such as cyclosporine, azathioprine, chlorambucil, or mycophenolate are used as a second line, usually when the patient does not respond to or tolerate glucocorticoids or has a severe life-threatening clinical presentation early in the disease's therapy (Viviano 2022).

To evaluate the clinical outcome, a period of approximately 1 month was chosen. This was established according to numerous studies and recommendations concerning the time that dogs require to exhibit a response to diet, which is generally between 2 weeks and 1 month (Allenspach, Wieland et al. 2007; Walker et al. 2013; Dandrieux 2016; Tolbert et al. 2022). Also, the initial CIBDAI classification score was related to the improved and non-improved cases, although usually the final CIBDAI score is calculated to analyse the treatment's response in comparison to the initial one (Collins 2013).

A significant percentage of SG1 (70,4%-57/81) improved in response to the new hydrolysed diet regardless of the addition of treatments, while only 29,6% (24/81) dogs did not respond positively. Overall, these results could be justified by the diet's palatability affected by the hydrolysis process of hydrolysed diets (Rudinsky et al. 2018), as well as the dog's unique reaction to diet. The owner's compliance following a restricted regime of diet, or drug medications may also have had an influence on the dogs' clinical outcome (Westermarck 2016). In

addition, the non-response to diet or combined therapies could have been due to a different diagnosis, including IBD, intestinal neoplasia, other GI illnesses, or extragastrointestinal diseases, which may have been overlooked. Notably, therapies associated simultaneously with diet may have affected these results.

In Group A, no addition of adjuvant therapies was made concurrently to the hydrolysed diet, to be specific no other variables were changed at the same time. Half of these dogs (50%-8/16) showed clinical improvement. Of the dogs with no ancillary treatments, 75% (3/4) of them had a positive response to diet. Consequently, this contributes to the proportion of FRE cases diagnosed in a referral setting (Dandrieux, Martinez et al. 2019). However, in the remaining dogs, attention should be noted that the clinical improvement might have benefited not only from the new diet implementation but also from the ancillary treatments already on course.

Moreover, the majority of improved dogs in Group A had an insignificant to mild disease severity (75%-6/8), and of the unimproved 50% (4/8) were classified with moderate illness. Conversely, no association was found between the initial CIBDAI classification of disease severity and the clinical improvement following a hydrolysed diet trial of these cases. Therefore, this does not support a possible theory that clinical improvement was dependent of initial CIBDAI scores. Also, there are no studies to compare with since no survey has used the initial CIBDAI scores solely to assess the clinical outcome.

Concerning Group B, of the 40 dogs that added supportive treatment alongside hydrolysed diet 80% improved. Nearly half of them were supplemented with cobalamin, which could have had a significant difference in the clinical outcome (Walker and McMahon 2021), as it improves absorption and decreases degradable body condition (Hall and Day 2017, Kather et al. 2020). In most dogs to which PBs were employed, other supportive therapies were also added, except for 2 cases. Therefore, it is impossible to evaluate PBs' effect combined with diet on clinical improvement. Furthermore, in the unimproved dogs the majority of cases were classified with moderate severity disease, which could be arguable that this could have been related to the negative outcome, however, no association was discovered between the two categories.

Interestingly, in dogs that implemented antibiotics at consultation, an initial classification of an insignificant disease was calculated in 2/3 of the unimproved cases. Therefore, it could be argued that these unimproved dogs may have worsen in clinical presentation possibly due to the negative consequences of antibiotics, including dysbiosis (Pilla 2020) or the duration or implementation of medication could have been insufficient for the assessment of a good outcome.

Of the dogs in which immunosuppressants were administered with diet, most cases (61,5%-8/13) improved while 38,5% (5/13) did not show any improvement. The study conducted by Marchesi et al. (2017) showed a fundamental difference between the group with solely drug therapy (prednisone) *versus* the group with a combination of drugs and diet, especially in moderate and severe cases of IBD which had a decrease in the frequency of relapses. In fact, a severe disease was the most prevalent classification among the improved cases in the present study (37,5%-3/8). This demonstrates the importance of the diet in the hypersensitivity to food allergens and diminishing intestinal inflammation (Guilford and Matz 2003). Consequently, Marchesi et al. (2017)'s report supports the high percentage of improved dogs treated with immunosuppressants combined with diet in this study. Additionally, of the unimproved dogs, the majority (3/5-60%) were classified with a moderate CIBDAI score at presentation, although this was not associated with clinical outcome. The minor duration of the immunosuppressive trial might have influenced this outcome, or the diet did not have any significant impact, however, this cannot be determined.

The absence of statistical association between variables (disease's initial severity and clinical improvement) stresses that CIBDAI does not seem to dictate the clinical response to medical treatments.

Although this study allowed the obtention of multiple data and relevant conclusions, some limitations could be considered and are assumed.

Firstly, the clinical activity score (CIBDAI) used in the present study was retrospectively applied to evaluate the severity of the disease at consultations, prior to the implementation of hydrolysed diet. Although CCECAI is a stronger indicator for long-term prediction when compared to CIBDAI (since it includes pruritis, albumin level and ascites) (Volkmann et al. 2017), due to the paucity and diversity of the available retrospective information, CIBDAI was found more appropriate. A prospective study evaluating CCECAI initial and final scores would have increased accuracy in evaluating the clinical improvement of dogs when transitioning to a new diet. This was not possible due to the retrospective nature of this study, hence, clinical and clinicopathological abnormalities were solely analysed at the first presentation.

Secondly, several treatments were prescribed concurrently to the diet, confounding interpretation as to what portion of the clinical response was attributable to the hydrolysed diet. This is applied to all subgroups of main treatments (supportive, antibiotics and immunosuppressants). Generally, a step-up method is frequently applied to treat dogs with mild-to-moderate illness, starting with a dietary trial followed by an antimicrobial trial or immunosuppressive drugs. Conversely, a step-down approach is used for dogs with moderate-to-severe clinical disease, in which, usually, combined therapy of diet, antimicrobials, and immunosuppressive

drugs is administered, followed by a gradual redraw of immunosuppressive drugs and antimicrobials in cases of positive response (Makielski et al. 2019). Therefore, idiopathic CD treatments seem to have a dynamic process according to literature and these results, as owner compliance, clinic availability of diagnosis techniques and practitioners' knowledge are frequently considered in the therapeutic approach of dogs with CD.

Finally, dogs that did not respond to a particular hydrolysed diet, may have responded to a different brand or other food alternatives, including homemade diets, hyperdigestible diets, novel-protein source diets, low-fat diets, hyperdigestible prebiotic-rich diets or fibre supplementation.

5. Conclusion

More than reinforcing the role of hydrolysed diet on the therapeutic approach of canine CD, this study highlights that a large proportion of general practitioners do not invest in a proper dietary trial, as more than half of referred dogs had never experimented a hydrolysed diet prior to these consultations. Even though several studies emphasise that a diet trial should be the first line of treatment in the empirical sequential therapy in idiopathic CD, there is still a high frequency of cases diagnosed with FRE in referral centres. The latter recommendation was followed by the leading clinicians supervising these cases in the three referral centres, in which a hydrolysed diet was implemented in all these cases.

A minor percentage had received antibiotics and immunosuppressants before the hydrolysed diet trial, stressing the need for a better standardised therapeutic approach to a case of canine CD among first-opinion practitioners.

This study also supports a significant proportion of prescribed PBs and a smaller one of antibiotics, especially in referral practice, which can suggest a higher awareness of the prejudicial effects of antibiotics, investing in other novel alternatives such as probiotics to firstly address CD.

Part 2 - The impact of diet reassessment in the medical management of refractory chronic enteropathy in dogs

1. Introduction and objectives

After excluding extragastrointestinal causes, infectious diseases, mechanical obstructions and neoplasia, sequential treatments are attempted to diagnose and treat canine CD. These include firstly, a dietary trial, and secondly, immunosuppressants or antibiotics trials, depending on histopathological findings (Cerquetella et al. 2020). Some CD cases do not respond to these empirical medical treatments, being considered refractory cases. In detail, these cases are diagnosed when the above-mentioned treatments are unsuccessful, and CD

persists (Benvenuti et al. 2021). Nowadays alternative approaches for refractory chronic enteropathy (RCE) have risen, namely FMT and PBs (Dandrieux 2016; Makielski et al. 2019). However, data regarding the management of these complex cases remains scarce.

Recognising that diet is a common day-to-day treatment approach with important benefits in dogs with CD (Kathrani 2021), the possibility of this therapy influencing not only FRE but also aiding in the management of a considered RCE deserves further investigation. Although in RCE, the role of multiple dietary trials has been questioned since frequently multiple hydrolysed diets have repeatedly been tried and failed to enhance clinical outcomes.

Focusing on cases of CD consulted for referral or second opinion consultation, this study aims to assess the impact of an additional alternative dietary transition in dogs with a considered diagnosis of RCE that have already been fed with a hydrolysed diet.

2. Material and Methods

2.1. Sample population

The sample population was obtained following the same methodology carried out for the first part of this study (Section 3 Part 1). The main database of dogs presented to referral or second opinion consultations with CD was used, in line with the inclusion and exclusion criteria already presented.

2.2. Study Group 2 (SG2)

For this second study, cases with the final diagnosis of canine RCE were selected. In detail, that included dogs that at some point, either on their first or subsequent consultation, had previous unsuccessful trials with a combination of hydrolysed diet, deworming, probiotics or antibiotics and immunosuppressants. If clinical signs persist and there was no response to one or more immunosuppressants, dogs were integrated into this category, forming Study Group 2 (SG2). In addition, information about definitive diagnosis by histopathology was collected from the selected RCE cases (SG2).

At some point, several dogs from SG2 underwent another diet change. Dogs were either transited onto a different commercial hydrolysed diet, a commercial novel protein source diet, a novel protein source homemade diet, a commercial prebiotic-rich/hyperdigestible specific diet or a commercial hyperdigestible non-hydrolysed diet. This dietary choice was further described according to the types of diets mentioned.

Dogs that exclusively changed diet were analysed separately from the ones that had their medical treatment adjusted or changed concurrently. For this purpose, they were divided into sub-groups: Group A and B, respectively. In the former group, the main immunosuppressant and antibiotic treatments already on course were specified, and in the latter group, any

treatments given or changed besides diet were distinguished (supportive, antibiotics or immunosuppressants). The supportive therapies specified included Cobalamin supplementation, FS (psyllium) and FMT.

The cases from SG2 that changed to an alternative diet were further characterised considering clinical response after one month. Figure 2 illustrates the study procedure.

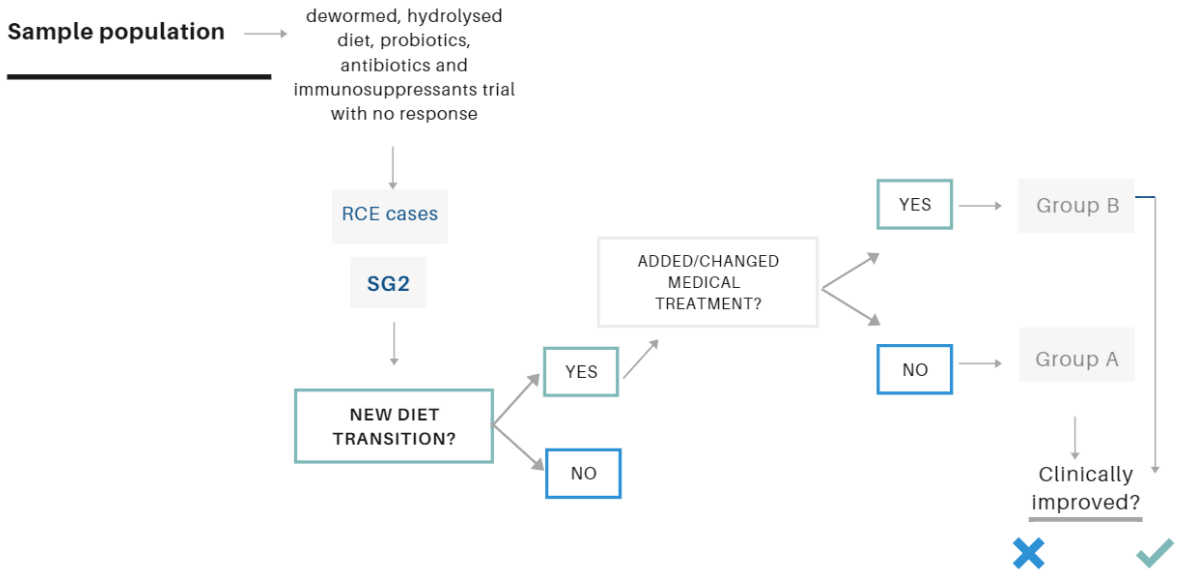


Figure 2- Study scheme and analysis of SG2

2.3. Data processing and statistical analysis

All data was collected and uploaded into a database using the software Microsoft® Excel® 2022, for statistical analysis. Only descriptive statistics were used, and the results were reported as absolute numbers and percentages (%).

3. Results

3.1. SG2 Characterisation

From the 142 dogs included in this study, 18,3% (26/142) were considered by the inclusion criteria as cases of canine RCE at some point during consultations (first consult or re-evaluations) forming SG2.

The SG2 was composed of 50% (13/26) each of female and male dogs. The median age was 6 (IQR=6,25) years old. Mixed breed dogs and Jack Russell Terriers were overrepresented with a prevalence of 15,4% (4/26) and 11,5% (3/26) respectively. Table 17 summarises the breeds delineated in this study.

Table 17- Breed characterisation of SG2

Breed	n	%
Mixed Breed/Undefined	4	15,4%
Jack Russell Terrier	3	11,5%
West Highland White terrier	2	7,7%
Golden Retriever	2	7,7%
Portuguese Water Dog	2	7,7%
Pomeranian Lulu-German Spitz	2	7,7%
German Shepherd	2	7,7%
Yorkshire Terrier	1	3,8%
Poodle	1	3,8%
Samoieda	1	3,8%
Weimaraner	1	3,8%
Shih-tzu	1	3,8%
Great Dane	1	3,8%
Siberian Husky	1	3,8%
Pitbull	1	3,8%
English Cocker Spaniel	1	3,8%

Legend: n of SG2= 26 dogs

In addition, from SG2 57,7% (15/26) had a histologic diagnosis of chronic lymphoplasmacytic enteritis, 12 obtained by endoscopy and 3 by laparotomy. Concerning the severity of GI inflammation, 3 were classified with mild severity, 7 with moderate, 4 with severe and another with moderate-severe enteritis. The remaining 42,3% (11/26) dogs had no histopathology available.

3.2. Dietary choice in new diet transition and treatments at presentation

Among these 26 RCE cases (SG2), 88,5% (23/26) were transitioned onto another alternative diet. More specifically, 16 dogs to a different commercial hydrolysed diet, one to a commercial novel protein source diet, 4 to a novel protein source home-made diet, a single dog to a commercial prebiotic-rich/hyperdigestible specific diet and another one to commercial hyperdigestible non-hydrolysed diet. Table 18 presents the types of diets and brands used in each category.

Table 18- Description of type of diets used in the RCE cases (SG2) that experiment a new diet

GROUPS OF TYPE OF DIETS	COMMERCIAL BRAND/ TYPE OF FOOD	n
COMMERCIAL HYDROLYSED DIET (n=16)	Purina HA ®	10
	Hills z/d®	4
	RC annalergenic®	2
COMMERCIAL NOVEL PROTEIN SOURCE (n=1)	Acana Duck®	1
NOVEL PROTEIN SOURCE HOMEMADE DIET (n=4)	Duck and Sweet potato	2
	Turkey, green beans, potato	1
	N/A	1
COMMERCIAL PREBIOTIC RICH/HYPERDIGESTIBLE SPCEFIC DIET (n=1)	Hills Biome®	1
COMMERCIAL HYPERDIGESTIBLE NON-HYDROLYSED (n=1)	RC GI medium callorie®	1

Legend: n of SG2 that implemented a new diet= 23 dogs; *N/A-not available;

3.2.1. Group A and B

The implementation of an alternative diet was the solo treatment measure in 69,6% (16/23) of dogs (Group A), while in 30,4% (7/23) an association of medical therapy was made concurrently with the dietary change (Group B).

3.2.1.1. Group A

Disregarding the ongoing treatments, including immunosuppressants, from the 16 dogs in Group A, 68,75% (11/16) transitioned onto another hydrolysed diet, 12,5% (2/16) onto a home-made diet, 6,25% (1/16) onto a prebiotic-rich/hyperdigestible specific diet, 6.25% (1/16) onto a commercial novel protein source diet, and lastly 6,25% (1/16) onto commercial hyper-digestible non-hydrolysed diet.

Of these dogs, concerning the immunosuppressant treatment, 10 cases had prednisolone being administered, another dog had cyclosporine, and the remaining 5 cases were under a combination of cyclosporine and prednisolone. In addition, 2 of these dogs were also under metronidazole, and another case was with sulfasalazine.

3.2.1.2. Group B

Of the 7 dogs in Group B, 71.4% (5/7) of cases switched to a different hydrolysed diet, whereas 28.6% (2/7) dogs switched to a homemade diet.

Regarding the dogs that changed to another hydrolysed diet (n=5), only one case switched immunosuppressant: the dog was being treated with azathioprine and changed to a

combination of prednisolone and cyclosporine. Also, one dog replaced its antibiotic choice for another (cefovecin to spiramycin) while maintaining the previously implemented methylprednisolone. Another dog was supplemented with cobalamin alongside the ongoing prednisolone treatment. Furthermore, one dog experimented a FMT as an adjuvant to the prednisolone treatment and the new diet. Moreover, in another patient, a FS (psyllium) was included in addition to the prednisolone.

Referring to the dogs that transitioned onto a homemade diet (n=2), both cases concurrently changed from prednisolone to cyclosporine medication.

3.3. Clinical outcome

In general, of SG2 that transitioned to another diet (n=23), 60,9% (14/23) had a favourable response to medical and dietary adjustment, while 39,1% (9/23) did not show any improvement.

From Group A (n=16), which exclusively changed diet, 68.8% (11/16) cases showed clinical improvement: 8 with a hydrolysed diet, one with a homemade diet, another with a prebiotic-rich/hyperdigestible specific diet and one with a commercial novel protein source diet. The remaining 31.3% (5/16) dogs did not improve with dietary change. Table 19 summarises Group A's clinical improvement according to the types of diet implemented.

Table 19- Group A's clinical improvement according to group of diets implemented

GROUP OF DIETS	CLINICAL IMPROVEMENT-GROUP A (n=16)	
	Improved (n=11)	Non-improved (n=5)
COMMERCIAL HYDROLYSED DIET (n=11)	8	3
COMMERCIAL NOVEL PROTEIN SOURCE (n=1)	1	0
NOVEL PROTEIN SOURCE HOMEMADE DIET (n=2)	1	1
COMMERCIAL PREBIOTIC.RICH/HYPERDIGESTIBLE SPCEFICI DIET (n=1)	1	0
COMMERCIAL HYPERDIGESTIBLE NON-HYDROLYSED (n=1)	0	1

Concerning Group B (n=7), in which medical treatment was changed simultaneously to diet, a clinical improvement was noted in 42.9% (3/7) of the patients, two of whom were fed with a hydrolysed diet and the third with a home-made diet. The other 57,1% (4/7) patients did not show a positive response. Table 20 describes Group B's clinical improvement according to the different types of diet used.

Table 20- Group B's clinical improvement according to the different groups of diet implemented

GROUP OF DIETS	CLINICAL IMPROVEMENT-GROUP B (n=7)	
	Improved (n=3)	Non-improved (n=4)
COMMERCIAL HYDROLYSED DIET (n=5)	2	3
NOVEL PROTEIN SOURCE HOMEMADE DIET (n=2)	1	1

4. Discussion

This study contributed to the assessment of the impact of dietary changes in the management of canine RCE that already tried hydrolysed diet in the past.

A selection of RCE cases was made from the sample population (18%) forming SG2. For the purpose of this study, cases were considered with a canine RCE in concordance with the literature, which argues that RCE is diagnosed when unsuccessful diet, antibiotics and immunosuppressants trials are experienced (Benvenuti et al. 2021). Although there is no clear definition on when to consider a RCE case, accounting for the immunosuppressants' attempts and the several options available, RCE was assumed at least after one failed immunosuppressant.

There was no sex overrepresentation in this study. Importantly, there is no literature regarding sex predisposition in RCE cases, although, in cases of CD and CE, males are usually overrepresented in myriad reports (Volkmann et al. 2017; Craven et al. 2004; Munster et al. 2006; Allenspach, Wieland et al. 2007; Allenspach, Curverlwell et al. 2016; Marchesi et al. 2017). Also, mixed-breed dogs and Jack Russell Terriers were overrepresented. Indeed, in a study led by Benvenuti et al. (2021), 22% (36/165) of dogs were of mixed breed in IRE or RCE cases, and in the research conducted by Kathrani et al. (2011), Jack Russell Terriers were overrepresented as well. Moreover, these cases were mostly middle-aged dogs, which is in line with several reports in cases of canine CE (Volkmann et al. 2017; Benvenuti et al. 2021).

Only 57,7% of SG2 dogs had a histologic diagnosis, all showing chronic lymphoplasmacytic enteritis, and most of them with a moderate severity. The remaining dogs had no information available regarding histopathology, either biopsies were not performed or not recorded. Indeed, it is only advisable the implementation of immunosuppressants after confirmation of intestinal inflammation with histopathology (Cerquetella et al. 2020), which was performed in those former 15 dogs with certainty. The lack of histopathology results can have been justified due to financial concerns, as well as ongoing immunosuppressants treatments which could have affected the histopathology results.

To author's best knowledge, there is currently no research assessing specifically the impact of diet in canine RCE cases. With exception of Marchesi et al. (2017), who demonstrated a dietary influence in cases treated with prednisone. However, in a significant percentage of SG2 (RCE cases) (88,5%-23/26), a decision of a new dietary transition was made in a referral context. In fact, Tolbert et al. (2022) argue that dietary intervention should be transversal across all dogs with CE, as it helps on decreasing food allergens, related to the intestinal inflammation.

Most dogs transitioned onto a hydrolysed diet (70%-16/23). This matches with Tolbert et al. (2022) proposal of the dietary approach to canine CD, which states that hydrolysed diets are the leading choice in cases of non-response to other specific diets (fibre-enriched, highly digestible, or low-fat diets). Also, this higher proportion using a new hydrolysed diet can also be justified by the clinician's preference or for its recognised benefits: it aids in decreasing intestinal inflammation by a reduction of food allergens, its possible low-fat content which can be beneficial especially in suspected cases of PLE, and the presence of omega-3 fatty acids and soy which have an immunomodulatory effect (Kathrani 2021).

Following hydrolysed diets, a minor percentage of dogs changed to a homemade diet (17%-4/23). As matter of fact, this diet is frequently used in cases of severe CE or RCE cases to improve a variety of food elements, as the commercial diets can lead to a decrease in appetite (Torres et al. 2003). In addition, these diets minimise the risk of cross-contamination of undeclared protein in commercial diets (Tolbert et al. 2022). In the study conducted by Luckschander et al. (2006), dogs fed with salmon and rice showed a reduction in CIBDAI scores after a 10-day treatment duration; however, these dogs were solely on diet.

One dog transitioned onto a commercial novel protein source diet, which in myriad reports have shown to improve clinical signs (Sauter et al. 2006; Allenspach, Wieland et al. 2007). Both hydrolysed and novel protein source diets are effective in dogs with CD, therefore the preference might rely on the acceptance of the dog or individual nutrient needs (Kathrani 2021). Another patient changed to a commercial prebiotic-rich/hyperdigestible specific diet, mainly rich in a fibre complex. These diets are generally used in dogs with signs of large bowel disease (Leib 2000; Tolbert et al. 2022). However, no information associated with the case was discovered related to these clinical signs.

Lastly, one dog was fed a commercial hyperdigestible non-hydrolysed diet. Notably, Tolbert et al. (2022) argued that highly digestible diets are usually used only in cases of mild small intestine disease and normal blood work. Also, in a study conducted by Mandigers et al. (2010), the highly digestible diet was able to induce remission in dogs with CE, although these patients were less likely to remain asymptomatic at succeeding re-evaluations compared with dogs fed with a hydrolysed diet. Nevertheless, this was only demonstrated in cases of CE or FRE, not specifically in RCE cases.

In general, the choice of diet can also have been according to previous unsuccessful diet trials, owner financial circumstances, market availability and owner compliance.

Diet was the single therapy altered in more than two thirds of cases (Group A). This might suggest a higher awareness regarding the unnecessary aggressive treatment options, which can destabilise the patient's condition and might not add any further benefit. Detailing these cases, most dogs were on prednisolone (62,5%-10/16), which is still considered a first-line immunosuppressant treatment (Viviano 2022), with a good short-term response (Dandrieux 2016). In one dog, cyclosporin was already being implemented, which has also been used in cases of PLE in dogs that do not respond to corticosteroid therapy, with a long-term improvement (3 years) in 70% (7/10) of dogs (Allenspach, Wieland et al. 2007). There is also an additional study that reported the use of cyclosporine in RCE cases, reporting a favourable response in 25% (1/4) dogs (Dandrieux, Noble et al. 2013). In this case, a transition to cyclosporine could have been required due to the patient's lack of response or intolerance to the effects of glucocorticoids (Viviano 2022). The remaining 5 dogs were already in a combination of these two immunosuppressants. There is a paucity of reports regarding this combination, however, it is described in the literature (Hall and Day 2017).

In Group B, most dogs were transitioned onto a hydrolysed diet, in which concurrent medical treatment was also adapted. One dog changed from azathioprine to prednisolone combined with cyclosporine. This could be justified by the adverse and severe effects of azathioprine, including myelosuppression (Hall and Day 2017) and lack of information regarding its value in CE (Dandrieux 2016). Additionally, one dog added another antibiotic to the previously implemented immunosuppressive therapy (methylprednisolone), more specifically spiramycin, which is an unusual choice for CE therapy. However, this antibiotic was probably used due to an oral concomitant lesion reported on this dog's medical records. Another dog on prednisolone was supplemented with cobalamin, as its cobalamin values were below the cut-off (400 ng/L) considered by Texas A&M University Gastrointestinal Laboratory (2018); hence this supplementation was indeed necessary. Furthermore, one dog with prednisolone experienced a FMT, which is one alternative therapy that has been recently applied in cases of an unsuccessful response to empirical treatments (Makielski et al. 2019). In fact, there are two reports of a positive response to FMT in RCE cases (Igarashi et al. 2014; Berlanda et al. 2010) supporting its potential benefit in these cases. Finally, psyllium was added to one case. An essential adjuvant in cases of large bowel disease, however, there is no indication on medical records that justify this supplement. Two dogs' diets were changed to a homemade meal and a concurrent transition from prednisolone to cyclosporine was made. It can be argued that this drug alteration might have been due to an unsuccessful trial with glucocorticoids (Viviano 2022).

Overall, of SG2 that transitioned to another diet, 39,1% (9/23) did not show any response to the new diet or to the therapy combination implemented. This puts into question if these are true cases of RCE since dogs could have had a different reaction to an alternative diet, to another immunosuppressants and alternative therapies such as FMT or PBs. However, an overlooked extragastrointestinal cause, infectious disease or intestinal neoplasia could have played a role in this poor outcome.

The majority of dogs from SG2 that changed diet (60,9%) had a positive response to the dietary modification. This is in agreement with Marchesi et al. (2017) who showed that diet had a significant influence in the clinical outcome of dogs treated with prednisone.

As for Group A, a substantial proportion of dogs with diet transition solely had a clinical improvement (68,8%-11/16). Indeed, if various nutritional approaches are attempted, there is a higher probability of finding the proper diet to manage these cases (Tolbert et al. 2022). In addition, most dogs with a hydrolysed diet reacted positively (73%-8/11) to this treatment addition, putting into question its role in RCE cases since its efficiency has been demonstrated in cases of CE (Marks et al. 2002; Mandigers et al. 2010; Walker et al. 2013; Allenspach, Culverwell et al. 2016; Wang et al. 2019). Another crucial point is that the duration and start of the therapies prior to consultation is unknown. Therefore, alongside the new diet, the immunosuppressants and antibiotics already ongoing could have also influenced this outcome

From the improved dogs in Group B that transitioned onto a hydrolysed diet, one of them changed diet simultaneously with a FS (psyllium) and another patient was supplemented with cobalamin. These supportive therapies could have had a favourable effect on the clinical outcome of these dogs; therefore, the impact of this diet is difficult to assess. In addition, from Group B, one of the two dogs fed with a homemade diet which changed from prednisolone to cyclosporine showed a favourable response. This result could have been due to the influence of the new immunosuppressant considering there is some evidence suggesting that cyclosporine is a good choice in a proportion of non-responders to glucocorticoids (Dandrieux 2016).

This retrospective study brought several innovative results, although there are some limitations that should be addressed.

Firstly, the use of different dietary choices for the new diet transition, as well as the different significant proportions of each type of diet, makes it challenging to compare the diet's efficiency in the RCE cases analysed. However, it is interesting to observe that dogs with several diet alternatives achieved clinical improvement.

Secondly, the multimodal treatments concurrently with diet also limits the true effect of diet management, in which the paucity of consistency and standardization in immunomodula-

tory treatment among patients might have affected treatment response and recurrence of clinical signs. Nonetheless, in cases that did not change treatment simultaneously (Group A), diet might have played a role in the positive outcome.

Thirdly, another limitation relies upon the small sample, although this reflects that RCE is indeed uncommon in practice. Also, considering the number of cases included and the different pathologists involved, as well as potential subjective scores on histopathological lesions severity, these were not analysed. However, it is known that RCE cases have been associated with dilatation of chyliferous ducts (Benvenuti et al. 2021), which could have been interesting to analyse in unimproved cases.

Furthermore, two studies recently reported low-grade lymphoma in dogs that underwent endoscopic assessment for GI signalment (Couto et al. 2018; Lane et al. 2018). In this survey, there is the possibility that low-grade lymphoma was included in this selected group that got through another diet transition, considering that neither immunohistochemistry nor a PAAR technique was performed in any of these dogs.

5. Conclusion

Although several studies have been discussing some alternatives to aid in the treatment of RCE cases, (Makielski et al. 2019), diet management has never been truly investigated in the context.

In this retrospective study, it was demonstrated that dietary reassessment was frequent in dogs with RCE, accounting for clinical improvement, not only in cases in which ancillary treatments were simultaneously adjusted or prescribed but also in most dogs to whom diet was the single treatment addition on these patients. Therefore, this study highlights that a novel alternative dietary trial should be considered before declaring a canine RCE case, and diet should be reassessed as it might have a crucial role in the therapy management of these cases.

Part 3- Final Discussion and Conclusion

1. Final Discussion

Considering the results of both studies, some observations should be discussed in a more comprehensive analysis.

There is a solid and unanimous consensus related to dietary trials as a first-line therapy in the management of CD (Tørnqvist-Johnsen et al. 2020). This principle was followed by board-certified specialists who supervised the referred cases that had never tried a hydrolysed diet before consultation. This suggests that first-opinion practitioners do not always perform an

appropriate dietary trial prior to referral since 57% of cases had never experienced a hydrolysed diet before. Notably, a good diet trial prior to referral is essential to avoid unnecessary aggressive treatment (Isidori et al. 2022).

In both studies, a hydrolysed diet was the leading choice either as a first-line or adjuvant therapy to canine CD as well as in RCE cases. In the first study, all dogs that had never tried a hydrolysed diet before referral (SG1) were all submitted to a dietary transition with a hydrolysed type, possibly due to its numerous benefits and proven effectiveness (Kathrani 2021). Interestingly, in the second study, even in the RCE cases which had already been fed with this specific category of diet prior to referral (SG2), the board-certified specialists insisted on another dietary transition onto a different hydrolysed diet. These findings enhance the scientific conviction of the role of diet in the management of canine CD, particularly in RCE cases.

In addition, the dietary impact was assessed in dogs with RCE (SG2), highlighting that those who changed diet, had an overall clinical improvement. This stresses that different nutritional approaches should reasonably be reconsidered in the management of CE and RCE cases, as suggested by Tolbert et al. (2022). Indeed, there is a lack of reports regarding a new dietary transition in cases of RCE. However, it has been shown that some dogs with FRE which had undergone dietary trials prior to referral still responded to an alternative diet (Dandrieux, Martinez et al. 2019), as well as dogs with PLE which successfully improved with a low-fat diet (Bota et al. 2016; Rudinsky et al. 2017).

Moreover, alongside dietary management, several drug combinations were administered in some cases from both studies. That included supportive therapy, antibiotics or immunosuppressants. These could have been prescribed in response to the dog's needs and owner's expectations, considering it is arduous to withhold drug therapy, especially in a referral consultation (Westermarck 2016). Notably, these medications could also have had an impact on the clinical outcome of dogs in this study.

Also, a great percentage of general practitioners, second-opinion veterinarians and specialists prescribed PBs, even though there is no real scientific consensus on their effectiveness in cases of CD (Dandrieux and Mansfield 2019). Actually, Jensen and Bjørnvad (2019) highlighted that diet has a greater impact than PBs. All things considered, practitioners appear to support its choice based upon PBs' beneficial properties and possibly to reduce unnecessary use of antibiotics in cases of suspect dysbiosis (Jensen and Bjørnvad 2019; Isidori et al. 2022). A prospective study is needed to evaluate the real effect of PBs solely on GI disease.

Remarkably, in both reviews, most cases revealed a positive response to the treatments proposed and to the new diet transition, combined or as a single therapeutic approach. It is plausible that clients who are prepared to pursue referral are more receptive to follow a strict diet experiment, which could support the eminent success after referral (Dandrieux, Martinez et al. 2019).

2. General Limitations

Overall, the retrospective nature of this study, transversal to the two analyses developed in Part 1 and 2, led to a less precise selection on account of some unfeasible information on definitive diagnoses. Also, in consequence of this retrospective review, it was not possible to standardise a diagnostic or treatment approach, as these were based on the severity of the clinical appearance, the doctor's clinical judgement, as well as consideration of the owner's financial and compliance restrictions at the time.

Additional key points regarding the selection of the sample population must be mentioned. Although dogs with diagnoses of extragastrointestinal causes or infectious diseases were excluded, some patients did not formerly rule out hypoadrenocorticism, pancreatitis, or infectious diseases, stressing the inability to clearly nominate these as canine idiopathic CD cases. These findings were also influenced by the retrospective nature of this study. Nevertheless, based on cost analysis and on a favourable response to empirical treatments, not all diagnostic approaches are necessary, depending on clinical presentation, analysis results, as well as owner compliance (Volkmann et al. 2017).

Equally important, the sample population could have been much more representative with a larger number of dogs. However, numerous cases were excluded considering the loss of follow-up and lack of owner feedback after diet transition. As a result, a more significant proportion of dogs could have been diagnosed with FRE in a referral context.

All collected data from the three referral centres were gathered onto the same database according to the inclusion and exclusion criteria, as well as supplementary information such as analysis results and clinical signs. However, it would have been interesting to compare the different approaches in the three referral centres from Portugal, Italy, and France with a more representative sample of each country, to evaluate the Europe perspective on the diagnosis and treatment approaches in cases of CD.

Furthermore, clinical improvement was not consistently observed at a re-evaluation consultation since it was a short period of follow-up (1 month), and the owner's perspective of improvement was the main measuring tool, which limits this evaluation. Additionally, the owners' memory of clinical signs and response to the treatments prescribed, as well as inaccurate medical history prior to these consultations, influence the perception of treatment duration and the exact response to diet and other medications. Also, the owner's compliance was not always recorded, which could have been helpful in understanding the actual course of therapy.

Finally, more prospective studies should be performed to evaluate the differences between hydrolysed diets and the other recognisable alternatives used for GI diseases. Also, an additional investigation should identify the ideal type and amount of diet that will aid in improving clinical signs in canine CD.

3. Conclusion and Future perspectives

This study emphasises the need to conduct a consistent dietary trial prior to referral in cases of canine CD, considering a hydrolysed diet had never been implemented by a significant proportion of first-opinion practitioners before referral or second-opinion consultations. Surprisingly, some of these dogs had already been administered with aggressive therapies, such as antibiotics and immunosuppressants, without ever trying a hydrolysed diet.

A large percentage of canine CD and RCE cases showed a clinical improvement as a result of the diet transition alone or alongside other therapy. Therefore, this study also highlights that several nutritional approaches should be attempted in the treatment of CD cases, considering that there are higher chances of selecting a more appropriate diet trial suitable to the patient's needs which can lead to a positive response. Notably, diet trials are still considered the first-line treatment approach in canine CD. However, dietary management appears to be an essential adjuvant to all empirical and combined therapy in cases of CD and RCE. This suggests that diet is a transversal component in all the disease's stages, not purely as a first approach.

Ultimately, the pyramid strategy should be reassessed, and a novel view of the empirical treatments should be developed, to enhance the significant role of the diet on CD cases, as well as to establish the appropriate treatment approach of antibiotics, PBs and FMT, especially in cases of dysbiosis and RCE cases.

Studies conducted on a larger scale, and in diverse veterinarian centres, would be useful to extrapolate these suggestions and bring a greater awareness around the importance of the diet in the management of all canine CD's cases, to the veterinarian community, allowing the creation of a standard clinical management approach.

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Section 5-Annexes

Annexe 1- Abstract 1 submitted to Congress of the European College of Veterinary Internal Medicine– Companion Animals 2022

The role of hydrolysed diet in the management of chronic diarrhoea in dogs – is it always the first therapeutic step?- A retrospective multicentric study

S. Duvergé[1], N. Santos[1], M. J. Dias[1-3], M. Hebert[4], E. Bettin[5], F. Signorelli[5], J. Hernandez[4], F. Procoli[5], R. O.Leal[1-3]

1. Veterinary Teaching Hospital, Faculty of Veterinary Medicine – University of Lisbon, Portugal

2. CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Vet Medicine – University of Lisbon

3. Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS)

4. Oniris Ecole Nationale Vétérinaire Agroalimentaire et de l'Alimentation – Nantes, France

5. Anicura Ospedale Veterinario I Portoni Rossi, Bologna, Italy

Diet has a major role in the therapeutic approach to canine chronic diarrhoea. In these cases, hydrolysed diet or novel protein diets are generally used as a first treatment step, with a good response and a better long-term outcome.

This study aims to evaluate the percentage of canine chronic diarrhoea cases presented for referral or second opinion consultation, to which a hydrolysed dietary trial was not previously performed, assessing the relevance of dietary change in those cases.
A retrospective multicentric study involving three referral centres was conducted. Medical records of dogs referred or consulted for second opinion presenting chronic diarrhoea (> 3weeks duration) between April 2018 and December 2021 were reviewed. Cases were included if there was a complete medical history and follow-up for at least one month. Data concerning supportive therapy (probiotics, antiemetics, gastric protectants or cobalamin supplementation), antibiotics and immunosuppressants prescribed prior to the consultation was assessed. Cases were excluded if an extra-digestive cause of chronic diarrhoea was suspected or confirmed. Dogs that had no previous history of a hydrolysed diet trial were further characterized. For data presentation, descriptive statistic was used.

From 142 dogs that met the inclusion criteria, 81 (57%) had never received a hydrolyzed diet prior to these consultations. A total of 38/81(47%) dogs were not under medication when consulted; while 24/81(30%) were on supportive therapy; 10/81(12%) were receiving antibiotics and 9/81(11%) were under immunosuppressants.
At first consultation, the transition onto a hydrolysed diet was the only treatment decision in 16/81 (20%) dogs. In the remaining 65/81(80%), a dietary trial was prescribed in association with either supportive therapy (40/65; 62%), antibiotics (12/65; 18%) and/or immunosuppressants (13/65; 20%).
Overall, a clinical improvement was documented in 57/81(70%) cases: 8 over 16 dogs exclusively treated with dietary trial and 49 over 65 with diet and ancillary treatment. In 24/81(30%) no response was observed: 8 cases over 16 to which diet trial was the only treatment and 16 over 65 from those treated with diet and ancillary treatments.

More than reinforcing the role of hydrolysed diet on the therapeutic approach of canine chronic diarrhoea, this study highlights that over half of dogs that consult at a referral center never experienced a hydrolysed dietary trial. A minor percentage had received immunosuppressants and antibiotics prior to a hydrolyzed diet, stressing the need of a better standardized therapeutic approach of a case of canine chronic diarrhoea among first-opinion practitioners.

Annexe 2- Abstract 2 submitted to Congress of the European College of Veterinary Internal Medicine– Companion Animals 2022

The impact of diet reassessment in the medical management of Refractory Chronic Enteropathy in dogs- A retrospective multicentric study

S. Duvergé[1], N. Santos[1], M. J. Dias[1-3], M. Hebert[4], E. Bettin[5], F. Signorelli[5], J. Hernandez[4], F. Procoli[5], R. O. Leal[1-3]

1. Veterinary Teaching Hospital, Faculty of Veterinary Medicine – University of Lisbon, Portugal

2. CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Vet Medicine – University of Lisbon

3. Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS)

4. Oniris, Ecole Nationale Vétérinaire Agroalimentaire et de l'Alimentation – Nantes, France

5. Anicura Ospedale Veterinario I Portoni Rossi, Bologna, Italy

The role of further dietary trials in the therapeutic management of canine refractory chronic enteropathy (RCE) is called into question since often hydrolysed diets have already been tried without clinical improvement.

The aim of this study is to assess the impact of further dietary change in dogs with RCE that have already been fed a hydrolysed diet.

Medical records of dogs presented with chronic diarrhoea (> 3 weeks duration) as a referral or second opinion consultation between April 2018 and December 2021, in three referral centres, were reviewed. Cases with RCE (defined as persistent diarrhoea after exclusion of extra-digestive and infectious causes and despite previous unsuccessful trials with a combination of hydrolysed diet, probiotics ± antibiotics, and immunesuppressants) were selected. Cases were further characterised based on dietary choice and clinical response over time.

In a total of 142 dogs with chronic diarrhoea, 26/142 (18.3%) were considered RCE. Of them, 15/26 (57.7%) had a histologic diagnosis of chronic lympho-plasmacytic enteritis while in 11/26 (42.3%) histopathology was not available.
Among RCE dogs, 23/26 (88.5%) were transitioned onto another alternative diet, either a different commercial hydrolysed diet, commercial novel protein source diet, novel protein source home-made diet, commercial prebiotic-rich/hyperdigestible specific diet or commercial hyperdigestible non-hydrolysed diet.
Dietary change was exclusively performed in 16/23 (69.4%): 11/16 (68.8%) switched for another hydrolysed diet, 2/16 (12.5%) for a home-made diet, 1/16 (6.3%) for a prebiotic-rich/hyperdigestible specific diet, 1/16 (6.3%) for a commercial novel protein source diet and 1/16 (6.3%) for a commercial hyperdigestible non-hydrolysed diet. From these, 11/16 (68.8%) showed clinical improvement: eight with hydrolysed diet, one with home-made diet, one with prebiotic-rich/hyperdigestible specific diet and one with a commercial novel protein source diet. The remaining 5/16 (31.3%) did not improve with dietary change.
Association of medical treatment was made concurrently with dietary change in 7/23 (30.4%). Apart from medical adjustment, 5/7 (71.4%) transitioned onto another hydrolysed diet while 2/7 (28.6%) onto a home-made diet. Clinical improvement was observed in 3/7 (42.9%), two fed a hydrolysed vs one a home-made diet. The remaining 4 dogs continued refractory to treatment.

Dietary reassessment was frequent in dogs with RCE, accounting for clinical improvement not only in cases in which concurrent treatments were simultaneously adjusted but also in most of those to whom diet was the single therapeutic measure. This study highlights that a novel dietary trial should be considered before declaring a canine chronic enteropathy refractory to treatment.