CANINE INTESTINAL DYSBIOSIS: A LITERATURE REVIEW

BY ÁINE SORCHA O'SHEA

Supervised by: Prof. Ágnes Sterczer

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LIST OF ABBREVIATIONS

GI   Gastrointestinal

FMT   faecal microbial transplant

BA   Bile Acids

UBA   Unconjugated Bile Acids

IBD   Inflammatory Bowel Disease

LPS   Lipopolysaccharide

GC-MS   Gas Chromatography-Mass Spectrometry

SIBO   Small Intestinal Bowel Overgrowth

CPSS   Congenital Portosystemic Shunt

QPCR   quantitative PCR

DI   Dysbiosis Index

SCFA   short-chain fatty acids

rRNA   Ribosomal RNA genetic material

RS   Resistant Starch

NRE   Non-responsive Enteropathy

IID   Idiopathic Intestinal Dysbiosis

ARD   Antibiotic Responsive Enteropathy

IRE   Immunosuppressant Responsive Enteropathy

IBD   Inflammatory Bowel Disease

FRE   Food Responsive Enteropathy

ABSTRACT

The primary objective of this literature review is to investigate contemporary research into canine intestinal dysbiosis and its importance in canine health today. A particular emphasis will be placed on the examination of metronidazole, tylosin, dietary intervention and probiotic therapy, with the aim of gaining enhanced insight into the current therapeutic approaches available to veterinary clinicians.

Understanding and effectively addressing canine intestinal dysbiosis is pivotal in promoting the optimal functioning of the canine digestive system, and linked extraintestinal disorders. The studies contributing to this literature review were systematically identified using PubMed, an esteemed online biomedical research repository, and were supplemented with scholarly material sourced from the University of Veterinary Medicine, Budapest. The work will review and discuss well-established research findings and give a balanced, comprehensive overview of current investigations in this field. Twenty studies were examined in their entirety, focusing on contemporary therapeutic approaches to various forms of intestinal dysbiosis.

This literature review found that modulations in the intestinal microbiome have been firmly established as being interconnected with general systemic health in dogs. Acquiring additional knowledge regarding this subject is of paramount importance to enhance the efficiency of applied clinical interventions in veterinary settings. Recent publications in the field have showcased significant advances in the understanding of shortcomings of commonly prescribed antibiotic therapies, whilst also highlighting a notable lack of consistency and coherence across research endeavours. Conversely, research discussed in this paper fortified the potential behind some dietary and probiotic interventions, indicating a promising outlook for futured studies into intestinal dysbiosis treatments. These statements are not intended to diminish any ongoing research efforts, rather their purpose is to underscore the inherent breadth of intestinal dysbiosis, and the intricate nature of approaching

this extremely complex issue. These findings emphasize the massive importance of additional research initiatives.

LITERATURE REVIEW

GUT MICROBIOME

GENERAL INTRODUCTION

Dogs have historically been labelled as a cherished companion of humans, hence earning the esteemed title of 'man's best friend'. With this special bond, arises the duty of safeguarding their well-being and a responsibility of ensuring optimal health and welfare. Recent research has demonstrated a growing imperative to understand gut microbial health and its consequential impacts (Alessandri et al., 2020 [2]; de Vos et al., 2022 [39]). Advancements in modern technology are facilitating a more comprehensive analysis of the constitution, diversity and detailed metabolic function of intestinal communities (Arnold et al., 2016 [11]; Kwa et al., 2023 [84]). Research in dogs has unveiled correlation between dysbiosis and: obesity (Ley et al., 2006 [92]; Kieler et al., 2017 [80]; Bermudez Sanchez et al., 2020 [21]), metabolic dysfunction (Montoya-Alonso et al., 2017 [109]; Jergens et al., 2019 [74]), cancer (Wu et al., 2009 [163]; Zitvogel et al., 2017 [166]; Pilla & Suchodolski, 2021 [121]) and even neurological issues (Wu et al., 2017 [162]; Gernone et al., 2022 [54]). This aids in proving that consequences of dysbiosis are not limited to being an intestinal tract issue. This paper aims to dissect previous 'gold standard therapies' and discuss recent dysbiosis research findings, with aim of gaining insight into obtaining optimal canine gut health.

CANINE GUT MICROBIOME

The microbiome describes the total population and genome collection complex of bacteria, archaea, protozoa, viruses and fungi found on and within a mammal's body (Pilla & Suchodolski, 2021 [121]). A product of approximately 500 million years of co-evolution

(Ley et al., 2008 [90]; Ley et al., 2008 [91]), the gut microbiome encompasses a diverse consortium of gastrointestinal microorganisms and their genomes, co-existing symbiotically in the GI tract (Hooper, 2001 [68]; Stecher & Hardt, 2008 [157]). This gastrointestinal microbiome assemblage vitally contributes to maintaining the health of the host organism. Extensive studies conducted on numerous mammalian species including human beings, have demonstrated the importance of the microbiome: in supporting homeostasis, metabolism, competitive exclusion of potentially pathogenic organisms, epithelial physiological maintenance, neuro-behavioural development and immunological function (Batt et al., 1996 [16]; Barko et al., 2018 [15]). Microbial genetics amassed within the biome, endow the host organism with an even greater metabolic repertoire than its own genome could provide, thereby supplying vital complimentary functions that are essential to host digestion (Barko et al., 2018 [15]; Goodrich et al., 2017 [55]). The microbiome is dynamic and therefore subject to a variety of factors including diet (Leverett et al., 2022 [89]), environment, stress (Pilla & Suchodolski, 2020 [120]), disease, medical intervention, dysbiosis and many different metabolic diseases (Pilla & Suchodolski, 2021 [121]).

In healthy dogs, the bacterial population within the gastrointestinal system typically spans between 10^{12} - 10^{14} cfu/g, a numerical magnitude approximately tenfold greater than that of the host cells (Suchodolski, 2011 [147]; Suchodolski, 2011 [148]). This ratio underscores the immense magnitude and physical presence of the gut microflora and their amassed genomes. This substantial statistic highlights the need for additional research in this field.
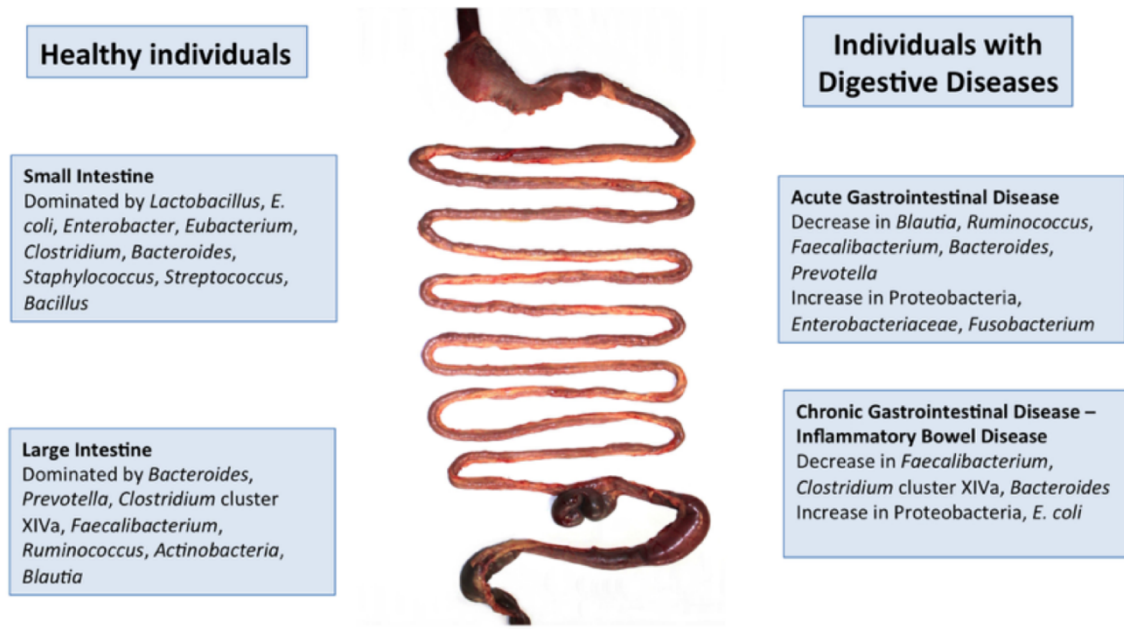
CANINE GUT MICROBIOTA

In comparison to the broader concept of the microbiome, the term microbiota specifically embodies all the distinct microorganisms that interact with a host in a particular environment (Grice & Segre, 2011 [56]). The composition of the gut biome exhibits remarkable diversity; consisting predominantly of bacteria, archaea and viruses. Among these components, bacteria, in particular (Suchodolski, 2011 [137]), play a significant role in essential digestive functions like fibre fermentation, pathogen protection and immune feedback for the host.

Ongoing research of microbiome functionality has uncovered important gut-link connections like the 'gut-brain axis' and gut-skin axis' (O'Mahony et al., 2015; O'Neill et al., 2016 [113]; Lee at al., 2018 [87]; Gernone et al., 2022 [54])

The predominant bacterial phyla found in the canine GI tract are Firmicutes and Bacteroidetes (Hoffmann et al., 2016 [64]). Other common phyla include: Proteobacteria, Actinobacteria, Spirochaetes, Fusobacteria, Tenericutes, Verrucomicrobia, Cyanobacteria and Chloroflexi. Within the firmicutes phylum, distinct bacterial groups like clostridia are identified. Clostridial clusters XIVa and IV are responsible for producing short chain fatty acids (SCFA). SCFA serve as an energy source once catabolized and are utilized by bacteria in the gut. They are believed to enhance barrier function, intestinal motility and reduce intestinal inflammation (Machiels et al., 2014 [98]). Bacteria convert fibre, protein, fat and bile acids into metabolites that can be more efficiently utilized by the intestine and other organs. SCFA, Indoles and secondary BA, are notable metabolites formed through microfloral processes (Bansal et al., 2010 [14]; Duboc et al., 2013 [44]; Pavlidis et al., 2015 [116]; Waclawikova & El Aidy, 2018 [168]; Minamoto et al., 2019 [105]).

Comparison of healthy and diseased intestinal microflora (Fig. 1: Adapted from: Hoffmann et al., 2016 [64])

Healthy individuals

Small Intestine
Dominated by *Lactobacillus, E. coli, Enterobacter, Eubacterium, Clostridium, Bacteroides, Staphylococcus, Streptococcus, Bacillus*

Large Intestine
Dominated by *Bacteroides, Prevotella, Clostridium* cluster XIVa, *Faecalibacterium, Ruminococcus, Actinobacteria, Blautia*

Individuals with Digestive Diseases

Acute Gastrointestinal Disease
Decrease in *Blautia, Ruminococcus, Faecalibacterium, Bacteroides, Prevotella*
Increase in Proteobacteria, *Enterobacteriaceae, Fusobacterium*

Chronic Gastrointestinal Disease – Inflammatory Bowel Disease
Decrease in *Faecalibacterium, Clostridium* cluster XIVa, *Bacteroides*
Increase in Proteobacteria, *E. coli*

Microbial populations along the GI tract exhibit variation. This is relative to the distinct microenvironment and functional role that is inherent to each intestinal segment. Bacterial data for the colon showed a broader range of 10^9-10^{11} cfu/g, indicating a more diverse range of flora compared to the small intestine (Suchodolski, 2011 [147]). Notably, the small intestine accommodates a blend of aerobic and facultative anaerobic bacteria, whereas the colon is mainly colonized by anaerobes (Pilla & Suchodolski, 2020[120]).

Through sequence analysis of 16 S rRNA (Pace, 1997 [114]), the intricate ecosystem within canine organisms has been unveiled, revealing the existence of at least 200 small-intestinal phylotypes and up to 1000 large-intestinal microorganism phylotypes (Handl et al., 2011 [58]; Handl et al., 2013 [59]) These findings have demonstrated some parallels to feline and human microbe tracts, and these complex intestinal microbes exert significant impact on both health and disease.

MICROBIOME ANALYSIS METHODS

Scientific interest in the microbiome can be traced back to the historic statement by Antonie van Leeuwenhoek [1632-1723] "I then most always saw, with great wonder, that in the said matter there were many very little living animalcules, very prettily a-moving." (Institute of Medicine, 2013 [71]). Records indicate that as early as 1977, scientists were cultivating the gastrointestinal tract in beagles, to make further microscopic deduction (Huang et al., 2020 [69]). However, the isolation of specific bacterial species in large quantities was very challenging, until PCR analysis methods were developed. Beneficial gut bacteria are predominantly anaerobic, therefore rendering traditional culturing methods ineffective in comprehensively assessing the full complexity of all bacteria in given sample (Costa & Weese, 2019 [31]). Bacterial culture is typically diagnostic only with relevant clinical signs such as: depression, apathy, visceral/parietal pain, dysentery, melena or an inflammatory leukogram (Marks et al., 2011 [102]; Werner et al., 2020 [173]; Becher et al., 2021 [17]). Although cultures are commonly taken from symptomatic dogs, diagnostic yield is low and false positives occur frequently. (Marks et al., 2011 [102]). More methods include gram staining of anaerobic bacteria, gas chromatography for fermentation-formed FA (Lin et al., 2022 [95]; Mackei et al., 2022 [99]) and other biochemical tests (Forster et al., 2018 [48]).

In 1993, the Denaturing Gradient Gel Electrophoresis (DGGE) technique was initially employed. DGGE serves as a tool to examine genetic variation within intricate microbial communities, including environmental or faecal samples (Muyzer et al., 1993 [110]). Through utilizing PCR-DGGE molecular fingerprinting, various faecal diversity levels can be found (Muyzer & Smalla, 1998 [111]). Technical biases associated with this technique are possible, and potentially can arise from such factors as template annealing during the amplification process (Suzuki & Giovannoni, 1996 [156]). Also of note, is that each single DGGE band does not necessarily represent a single bacterial strain, and so can be misleading (Sipos et al., 2007 [144]).

Another method of analysing bacterial community profiles is through percent G + C profiling (Apajalahti et al., 2001 [10]). This technique analyses the composition of bacterial

communities based on the relative abundance of guanine (G) and cytosine (C) DNA nucleotides. G+C content varies among different bacterial species, hence analysing these profiles allows researchers to identify and classify bacteria present. By applying percent G+C profiling to canine microbiome research, scientists gain a better understanding of the diversity and composition of the gut. However, this technique tends to hold bias for high G+C-containing strains (Suchodolski, 2011 [147]) so could perhaps be better used in combination with DGGE. Using the modified GC-DGGE technique improves diversity and minority microbiome assessment within faecal samples (Holben et al., 2004 [65]).

Microbial culture, while useful for culturable bacteria, has been largely replaced by molecular methods, due to their ability to capture non-culturable bacteria (Suchodolski, 2011 [147]; Suchodolski, 2011 [148]). 16S rRNA gene sequencing and shot-gun sequencing are molecular methods that identify species diversity in a presented sample. These methods involve fragment amplification and sequencing in the 16S rRNA conserved gene region, or work through sequencing all the available sample DNA. Costly shotgun sequencing holds the benefit of precisely coding functional genes, not just identifying the bacteria type. qPCR is a reliable quick, and cost-effective means of quantifying taxa that are of medical interest (Pilla & Suchodolski, 2021 [121]).

One prevailing limitation in many microbiome studies is the common practice of comparing a control group or baseline to various environmental factors like geography, diet, specific breeds and sample storage (Pilla & Suchodolski, 2021 [121]) Particularly when this occurs in a small size study, it becomes difficult to ascertain the complete extent of a single, specific variable.

CORE CANINE MICROBIOTA

The gut microbiome comprises bacteria, archaea, viruses, and eukaryotic organisms, all residing in the gastrointestinal (GI) tract (Thomas et al., 2022 [161]). The largest component within the microbiome is the bacteria, fulfilling a vital role in digestive processes, notably the fermentation of fibres (Swanson et al., 2011 [158]). Moreover, the gut microbiome lends

to host metabolism, immune system regulation and protection against pathogens (Pilla & Suchodolski, 2021 [121]). Advances in understanding microbiome functions have uncovered various connections the gut microbiome holds outside of the gastrointestinal tract. These have led to the coinage of terms such as the gut-brain axis, gut-skin axis, and so on (Lee et al., 2018 [87]; Pilla & Suchodolski, 2021 [121]; Gernone et al., 2022 [54]).

INTESTINAL DYSBIOSIS

Intestinal dysbiosis, characterized by an imbalance in the gut microbial community, is a prevalent condition that affects dogs of all breeds and ages. Various factors including dietary and medical interventions can disturb the delicate equilibrium between beneficial and harmful, leading to gastrointestinal distress and associated health combinations (Hooda et al., 2012 [67]). Numerous diseases, whether manifested systemically or in localized regions, have been correlated with dysbiosis (Pilla & Suchodolski, 2020 [120]). In essence, intestinal dysbiosis concerns alterations in the composition of the gut microbiome, that affect its function. (Zeng et al., 2017 [161]). Dysbiosis involves a shift in bacterial abundance or a reduction in species diversity, which in the case of the intestinal form, results in a metabolite dysmetabolism (Minamoto et al., 2019 [105]; Blake et al., 2019 [24]). It is important to emphasize, that owing to such colossal numbers of intestinal bacteria, a shift in bacterial populations is incredibly difficult to directly induce. Consequently, dysbiosis frequently assumes a chronic nature, involving physiological mucosal alterations (Ziese & Suchodolski, 2021 [162]). The increase in abundance of facultative anaerobic bacteria of the family Enterobacteriaceae is a hallmark of dysbiosis across multiple species, including dogs (Vazquez-Baeza et al., 2016 [165]; Rivera-Chavez et al., 2017 [129]).

Metronidazole and tylosin, both commonly prescribed antibiotics, have been widely used as a therapy for canine dysbiosis in the veterinary field. Both carry broad spectrums of action targeting gram – and +, anaerobic bacteria and some protozoal species. However, recent research has identified that these previously common therapies, may not be as efficient as hoped (Shmalberg et al., 2019 [142]; Langlois et al., 2020 [85]).

DYSBIOSIS INDEX

A commercially available PCR-based assay tool which assess individual canine and feline GI microbiome, with a 74% sensitivity and 95% specificity (AlShawaqfeh et al., 2017 [8]; Sung et al, 2022 [155]). DI is a method for evaluating GI microbiota providing an insight to intestinal disease, but also a method of evaluating treatment response. Currently it is the only analytically validated PCR assay available and has been employed in many published clinical studies, in both dogs and cats (Giaretta et al., 2018 [52]; Guard et al., 2019 [51]; Pilla et al., 2020 [118]; Sung et al., 2022 [155]). As previously discussed, there are thousands of canine GI microbiota, but DI focuses on quantifying the faecal abundance of seven bacterial taxa: Faecalibacterium, Turicibacter, Blautia, Fusobacterium, Bifidobacterium, Bacteroides, Clostridium hiranonis, Streptococcus and E. coli. Immunochemistry or in situ hybridization evaluates and identifies intracellular and muco-adherent bacteria in animals with GI disease (Suchodolski, 2021 [149]). Clear reference intervals are set for dogs where deviations have been linked to dogs with gastrointestinal disease.

Dysbiosis Index reference intervals for dogs *(Fig. 2: Adapted from: Texas A&M University, 2023 [160])*

	Function	normal in Dogs	Change in dysbiosis
Faecalibacterium	anti-inflammatory, production of SCFA	3.4 – 8.0	↓
Turicibacter	production of SCFA	4.6 – 8.1	↓
Blautia	production of SCFA	9.5 – 11.0	↓
Fusobacterium	production of SCFA	7.0 – 10.3	↓
Bifidobacterium	production of SCFA	not measured	↓
Bacteroides	production of SCFA	not measured	↓
Clostridium hiranonis	conversion of primary to secondary bile acids	5.1 – 7.1	↓
Streptococcus	overgrowth associated with dysbiosis	1.9 – 8.0	↑
E. coli	pro-inflammatory	0.9 – 8.0	↑

By evaluating the richness of the bile acid transforming bacterium *Clostridium hiranonis*, the intestinal microbiota's ability to transform primary to secondary BA is also evaluated by the DI. Secondary BA when in adequate numbers, play an antimicrobial role and suppress possible enteropathogens, such as C. difficile (Weingarden et al., 2014), C. perfringens (Blake et al., 2020 [23]), and E. coli. Consequently, a reduction of C. hiranonis and a decreased conjugation of primary BA strongly supports a faecal dysbiosis diagnosis. An elevated DI coupled with a decreased abundance of C. hiranonis, are commonly seen markers in many intestinal and extraintestinal disorders.

*Extraintestinal disorders that can lead to GI clinical symptoms (**Fig. 3**: Adapted from the lecture notes of Dr. K. Pápa, 2023)*
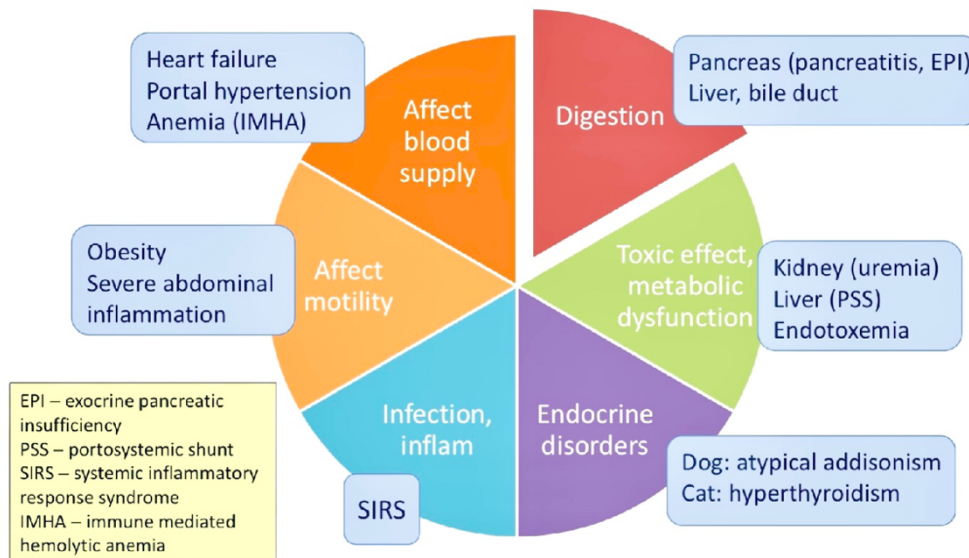
The qPCR method behind DI, uses the closest centroid classifier to calculate a single numerical value in relation to the mean prototype of each bacterial class. Therefore, a negative DI value suggests normobiosis; and conversely a positive DI value implies dysbiosis and a lack of bacterial richness in a sampled host (AlShawaqfeh et al., 2017 [8]). Really high DI, above 2 in dogs, can indicate marked intestinal mucosal deterioration and refractory GI disease (AlShawaqfeh et al., 2017 [8]; Mishima & Sartor, 2020 [108]; Suchodolski, 2023

[151]). Toxin detection is applicable for some bacteria such as Clostridium or pathogenic E. coli.

## DYSBIOSIS IN DIFFERENT DISEASES

ENTEROPATHIES & INTESTINAL DISEASE

Chronic enteropathy (CE) in dogs describes a group of non-specific diseases, characterized by gastrointestinal signs that involve chronic inflammation of the GI tract, and consequential GI signs that persist for atleast 3 weeks (Simpson & Jergen, 2011 [143]; Dandrieux, 2016 [33]; Hall & Day, 2017 [57]). The pathogenesis of canine CE is characterized by numerous contributing factors, with the foremost being a disruption in typically diet—related antigen tolerance or the intricate microbiome balance. These factors result in an aggressive cell-mediated immune response (Allenspach & Mochel, 2021 [5]). Due to its complicated nature, diagnosing CE necessitates ruling out other diseases that may present similarly, such as: bacterial infection, endoparasites, EPI, atypical hypoadrenocorticism and GI neoplasias (Berghoff & Steiner, 2011 [20]; Simpson & Jergens, 2011 [143]; Allenspach et al., 2016 [4]; Dandrieux , 2016 [33]). Common clinical signs include abdominal pain, chronic intermittent/persistent diarrhea, vomiting, cachexia, anorexia, borborygmus and nausea (Schmitz et al., 2015 [139]; Procoli, 2020 [123]).

Further classification of CE is possible, listed here in order of high occurrence: Food-Responsive Enteropathy (FRE), Immunosuppressant Responsive Enteropathy (IRE) Idiopathic Intestinal Dysbiosis (IID) and Non-Responsive Enteropathy (NRE) (Jergens & Heilmann, 2022 [75]). Previously IID was known as Antibiotic Responsive Enteropathy (ARE). Often further classification of CE is retrospective to treatment trial response e.g. dogs with symptom improvement on elimination diets are categorized as FRE. Upto 2/3 of CE presenting cases are connected to a FRE (Craven et al., 2004 [32]; Allenspach et al., 2007 [7]) although more recent research, involving the new disease categorization is warranted. Cases requiring corticosteroids for patient improvement are labelled as IRE, of which

sometimes can be termed Inflammatory Bowel Disease (IBD) (Jergens & Heilmann, 2022 [75]) NRE embodies the smallest proportion of CE cases, where multiple therapies have been applied with no notable response. An NRE diagnosis carries a poorer long-term prognosis and has been correlated with a high euthanasia rate (Craven et al., 2004 [32]; Allenspach et al., 2007 [7]). The legitimacy of true ARE has been subject to debate (Erdmann & Heilmann, 2017 [47]; Dandrieux & Mansfield, 2019 [34]; Cerquetella et al., 2020 [27])

Enteropathy Pyramid (Fig. 4*: Adapted according to Jergens & Heilmann (2022 [75]) & Dr. K. Pápa lecture notes (2023))*
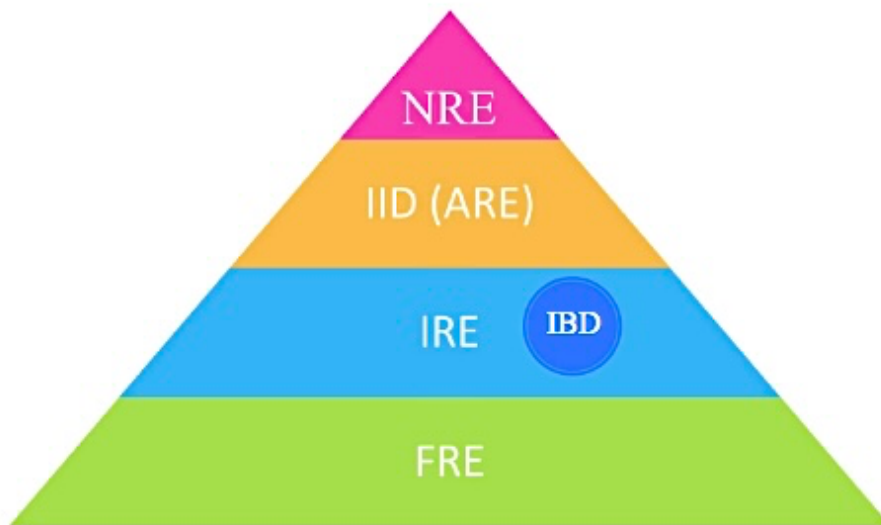
Clinical, pathological, endoscopic and histologic characteristics can resemble one another in dogs experiencing different types of CE. Definitive diagnosis of CE necessitates histologic evaluation of endoscopic or surgically obtained intestinal tissue biopsies (Elwood, 2005 [46]; Jergens et al., 2016 [77]). Immunohistochemical or other molecular tests may also be employed. The current consensus regarding the diagnostic CE algorithm in dogs, involves conducting elimination dietary trials ahead of more invasive testing methods (Jergens & Heilmann, 2022 [75]). Thus, dogs exhibiting complete and durable response to a vet-mediated elimination diet, may be classified as FRE, and not require further diagnostics (Holmberg et al., 2022 [66]). There are only a few secondary biomarkers, such as serum

cobalamin, albumin and faecal calprotectin, that hold clinical utility in CE presenting cases, and these need to be further confirmed based on various treatment response (Heilmann et al., 2018) [61]. Faecal calprotectin concentrations can be suggestive of IRE (Heilmann et al., 2012 [62])

Dysbiosis will always develop with chronic enteropathy. The immune system, genetics, food intolerance, intestinal dysbiosis and wall integrity are all suspected in the aetiology of CE (Allenspach, 2011 [3]; de Souza & Fiocchi, 2016 [38]). A breed link has been established: German Shepard (Chronic inflammatory enteropathy, EPI), Yorkshire terrier (Lymphangiectasia), Retrievers (Food responsive enteropathy, FRE), Border collies (Chronic Inflammatory Enteropathy), Boxer/French bulldog (Granulomatous Colitis/Histiocytic Ulcerative Colitis HUC).

Acute haemorrhagic diarrhoea syndrome AHDS is caused by Cl. perfringens overgrowth. Bacterial overgrowth: Campylobacteriosis, salmonellosis, coronaviral enteritis, pantropic coronaviral enteritis or miscellaneous bacterial enteritis. Young dogs in stressful environments often with concurrent disorders. Immunocompromised animals or dogs fed with raw meat diets are more prone to developing this condition and have a significant carrier state.

IATROGENIC DYSBIOSIS

Intestinal dysbiosis may be diagnosed through various clinical assessments and scoring systems and validated clinical scoring systems. These diagnostic tools serve not only to initiate appropriate treatment but also to identify the associated clinical consequences of intestinal dysbiosis.

CIBDAI

Canine Inflammatory Bowel Disease Activity Index (Jergens et al., 2003 [76]) is a systematic scoring assessment tool that was developed to help evaluate dogs suffering with IBD, which can further be validated through objective laboratory and histology indices that are suggestive intestinal inflammation. Through a 0-3 scoring system, individualized assessment is possible, depending on normal (0) to severe (3) salient clinical presentations of: activity, appetite, vomiting, stool consistency, stool frequency and observed weight loss. Hypoalbuminemia, hypocobalaminemia, hypovitaminosis D, duodenal lesions and an elevated CIBDAI score are all acknowledged as adverse prognostic indicators for canine CE (Jergens et al., 2003 [76]; Allenspach et al., 2007 [7]; Allenspach et al., 2017 [6]).

CCEAI

The Canine Chronic Enteropathy Activity Index scoring system is a more contemporary, validated approach for assessing the severity of dogs presenting with chronic enteropathy. Utilizing factors identified by logistic regression and ROC curve analysis, a clinical scoring index (CCEAI) was formulated to forecast adverse outcome in dogs suffering from chronic enteropathies (Allenspach et al., 2007 [7]). Severe disease is suggested at scores greater than 12/27. Hypocobalaminaemia, hypoalbuminaemia, hypovitaminosis D and an elevated CRP are also considered poor prognostic markers (Benvenuti et al., 2021 [19])

Comparison of clinical indices CIBDAI and CCEAI (Fig. 5: Adapted from Allenspach et al., 2007 [7])

CIBDAI

Attitude/activity
0 normal
1 slightly decreased
2 moderately decreased
3 severely decreased

Appetite
0 normal
1 slightly decreased
2 moderately decreased
3 severely decreased

Vomiting
0 normal
1 mild (1× per week)
2 moderate (2–3×/wk)
3 severe (>3×/wk)

Stool consistency
0 normal
1 slightly soft feces
2 very soft feces
3 watery diarrhea

Stool frequency
0 normal
1 slightly increased
   (2–3×/d) or fecal blood,
   mucus or both
2 moderately increased
   (4–5×/d)
3 severely increased
   (>5×/d)

Weight loss
0 none
1 mild (<5%)
2 moderate (5–10%)
3 severe (>10%)

CCEAI

Attitude/activity
0 normal
1 slightly decreased
2 moderately decreased
3 severely decreased

Appetite
0 normal
1 slightly decreased
2 moderately decreased
3 severely decreased

Vomiting
0 normal
1 mild (1×/wk)
2 moderate (2–3×/wk)
3 severe (>3×/wk)

Stool consistency
0 normal
1 slightly soft feces
2 very soft feces
3 watery diarrhea

Stool frequency
0 normal
1 slightly increased (2–3×/d) or
   fecal blood, mucus, or both
2 moderately increased
   (4–5×/d)
3 severely increased (>5×/d)

Weight loss
0 none
1 mild (<5%)
2 moderate (5–10%)
3 severe (>10%)

Albumin levels
0 albumin >20g/L
1 albumin 15–19.9 g/L
2 albumin 12–14.9 g/L
3 albumin <12 g/L

Ascites and peripheral edema
0 none
1 mild ascites or peripheral
   edema
2 moderate amount of ascites/
   peripheral edema
3 severe ascites/pleural effusion
   and peripheral edema

Pruritus
0 no pruritus
1 occasional episodes of itching
2 regular episodes of itching,
   but stops when dog is asleep
3 dog regularly wakes up
   because of itching

TREATMENTS: EFFECT & INFLUENCE ON THE INTESTINAL MICROBIOME
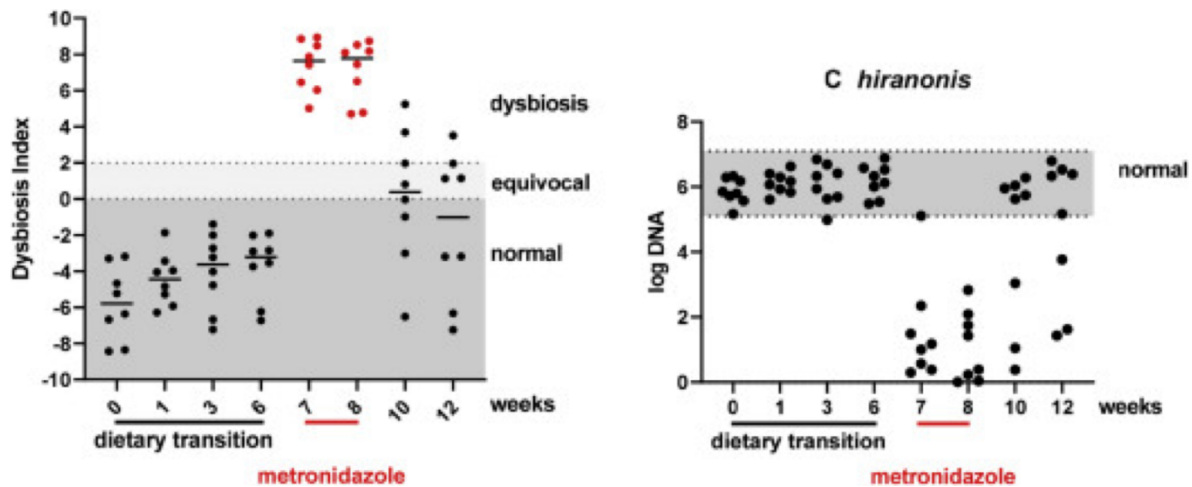
METRONIDAZOLE

Metronidazole, a bactericidal antibiotic of the nitroimidazole family, has been frequently prescribed in both human and veterinary medicine since the 1960s (Li & Corey, 2013 [94]). Despite over 60 years of research, the precise metronidazole metabolism and its associated cytotoxicity have yet to be definitively elucidated (Dingsdag & Hunter, 2018 [41]). Additionally, the post-treatment recovery of the microbiome, and the metabolic consequence have not been sufficiently investigated thus far (Pilla et al., 2020 [118]).

Nitroimidazoles, such as metronidazole, function as prodrugs that undergo activation through the reduction of the nitro-group. Research has demonstrated that it is the nitro-group that is responsible for the cytotoxicity (O'Brien & Morris, 1971 [112]; Ehlhardt et al., 1987 [45]). Around 45-70% of canine diarrhoea cases, are estimated to be treated with an antimicrobial prescription (German et al., 2010 [53]; Jones et al., 2014 [79]; Anholt et al., 2014 [9]; Radford et al., 2011 [125]; Singleton et al., 2017 [141]). Up until very recently, Metronidazole was commonly employed as a standard treatment in inflammatory infections of the GI tract, particularly in cases of: Acute Diarrhoea, colitis, giardia, C. perfringens (Singleton et al., 2019 [140]).

Recently, broad-spectrum antibiotics such as tylosin or metronidazole have been proven to reduce C. hiranonis and cause a subsequent decrease in secondary BA conversion. This directly causes a proportional increase in primary BA which has been linked to long-lasting subclinical dysbiosis in some dogs (Pilla et al., 2020 [118]; Chaitman et al., 2020 [28]; Manchester et al., 2019 [100]) (Fig. 6: Pilla et al., 2020 [118]; Ziese & Suchodolski, 2021 [161]). Fig.6 visually demonstrates how treatment with metronidazole can lead to an increased dysbiosis index, and the affected correlation of C. hiranonis numbers in the gut.

Metronidazole & Dysbiosis Correlation (**Fig. 6:** "Impact of Changes in Gastrointestinal Microbiota in Canine and Feline Digestive Diseases", Ziese & Suchodolski (2021 [161]) which was adapted from "Effects of metronidazole on the fecal microbiome and metabolome in healthy dogs" Pilla et al., 2020 [118])

Up until recent studies highlighted treatment detriments, metronidazole was the most commonly prescribed treatment for dogs suffering with acute diarrhoea, often associated with a suspected Giardia or C. perfringens infection (Singleton et al., 2019 [140]). C. perfringens is suspected of being the causative agent behind acute haemorrhagic diarrhoea syndrome in dogs, due to the strong netF toxin gene association (Leipig-Rudolph et al., 2018 [88]). However, a clinical trial has demonstrated no clear benefit of antimicrobial treatment in these cases, although metronidazole itself was not examined (Unterer et al., 2011 [164]). The study further stressed that as well as no demonstrated requirement for antimicrobial treatment of haemorrhagic diarrhoea in dogs, that continued unsubstantiated use of antibiotics is promoting their resistance, and unnecessary adverse drug reactions.

Similarly to acute course treatments, metronidazole is frequently administered to dogs with chronic diarrhoea upon failure to respond to dietary changes (Jergens et al., 2010 [73]; Rossi et al., 2014 [132]). Due to concerns of antimicrobials inducing dysbiosis in dogs with an existing deranged microbiome, alternative management via probiotics and synbiotics have

been projected (Rossi et al., 2014 [132]; White et al., 2017 [176]; Pilla et al., 2019 [119]). Additionally, it is difficult to ascertain if metronidazole administration alone affects microbiome composition or if dietary changes such as hydrolysed protein-based diets contribute too. Most cats and dogs with gastrointestinal (GI) disease suffer from concurrent intestinal dysbiosis (Alshawaqfeh et al., 2017 [8]; Marsillo et al., 2019 [103]). Dysbiosis patterns typically differ between acute and chronic diseases, however certain similarities persist across all conditions. The causative nature of dysbiosis remains unclear in most cases, with uncertainty as to whether the dysbiosis caused is a primary issue or a secondary effect of an underlying disease process (Ziese & Suchodolski, 2021 [161]). Furthermore, how much dysbiosis contributes to the extent and progression of a disease, remains uncertain in these induced secondary cases. Clinical manifestations resulting from dysbiosis are also likely influenced by the exact localization and degree of microbial change, therefore a massive variability in physical symptoms can be exhibited (Jalanka-Tuovinen et al., 2011 [72]).

Limited information exists regarding the impact of antimicrobial-induced dysbiosis on the serum and faecal metabolome, especially in dogs. Hence there is a need for more comprehensive research studies into microbiome and their functional-metabolite alterations. Pilla et al. (2020 [118]), explored the of a 14-day course of metronidazole, alone, or in conjunction with a hydrolysed protein diet. impact on faecal microbiota, microbolome and BA concentration. 3 groups were involved: group 1 acted as a control, group 2 received a hydrolysed protein diet in conjunction with metronidazole therapy, and group 3 received metronidazole alone. The evaluation of microbiome composition involved 16S rRNA gene sequencing and a qPCR-based dysbiosis index. Additionally, untargeted metabolomic analysis was conducted on faecal and serum samples, followed by targeted assays for faecal BA and lactate. Results for group 1 and 2 were mostly insignificant, however the application of metronidazole in group 3 displayed a notable alteration in microbiome composition, encompassing a reduced richness of microflora. These changes of the microbiota were found to persist 4 weeks past discontinuation of the antibiotic. Metronidazole administration in this present study, also demonstrated an increased abundance of Streptococcus and E. coli in samples. In correlation, the faecal dysbiosis index demonstrated a significant increase, along

with an increase in total faecal lactate levels. Results also demonstrated a shift towards primary BA, with a notable decrease in secondary deoxycholic and lithocholic acid. This study revealed Metronidazole held a minimum disruptive effect on faecal microbiota of a at least 4 weeks, further studies into the exact time-frame of effect and negative implications are justified.

Rudinsky et al. (2022 [133]) performed a 30-day dietary trial, involving 59 privately owned dogs diagnosed with non-infectious acute colitis. Experimentally, the goal was to examine the effects of dietary changes with or without metronidazole, through a randomized controlled clinical trial. The dogs were randomly assigned to 3 placebo-controlled groups, and all groups received an easily digestible diet for the duration of the trial. On top of this, Group 1 received a placebo tablet, Group 2 a metronidazole tablet, and group 3 received psyllium additions to their diet. The progress of the animals was then monitored through faecal scoring, remission time and the dysbiosis index. Median remission times across the groups were statistically different; with the metronidazole prescribed group displaying the longest time of 8.5 days. In addition to the remission times, metronidazole negatively influenced the faecal dysbiosis index during days 7 to 10. There is a need for more longitudinal clinical trials, in order to discern the comparative long-term responses, Stability and complications of metronidazole treatment for acute colitis in dogs.

An earlier study conducted by Igarashi et al. (2014 [70]) has also contributed to the enhanced understanding of the effects of metronidazole therapy on gastrointestinal microbiota. The study specifically examined the comparative impact of 14-day treatment of healthy dogs with either metronidazole or prednisolone. Both metronidazole and prednisolone are frequently prescribed treatments for dogs afflicted with various gastrointestinal disorders, including acute diarrhoea and chronic enteropathy. The study ran a duration of 42 days, and encompassed a cohort of 10 healthy beagle subjects, evenly divided into two groups: one receiving metronidazole treatment and one receiving prednisolone. Variations in age, gender and neutering status were present, but all dogs were housed separately in the same laboratory unit, fed identical diet and demonstrated no signs of gastrointestinal abnormalities as

determined by haematology, faecal examination and ultrasound techniques. Throughout the study, faecal samples were collected from each dog on day 0, 14 and 42. These samples were analysed through 16S rRNA gene sequencing. This approach facilitated comprehensive characterization of faecal microbial alterations over the course of the study period. Overall, the data suggests that metronidazole induced changes in faecal bacterial composition, leading to a reduction in bacterial diversity. Conversely, prednisolone was found to exhibit no similar effect. It is noteworthy that the impact of metronidazole in this study was found to be temporary in nature, diminishing in the 4-week span following the discontinuation of the drug. The results were valuable, but a more extensive sample size and perhaps an increase in breed representation and gastrointestinal disease incidence would have elicited scholarly curiosity. Also, the alterations in faecal bacterial groups could not necessarily be linked to clinical outcomes. Consequently, forthcoming research should employ canine study populations afflicted with chronic enteropathy.

As elucidated earlier, canine dysbiotic disruptions can lead to a myriad of subsequent health implications for dogs. However, following study, proposes the converse to also be true., further highlighting the importance of managing canine gut health. Metronidazole, a medication often employed in the treatment of dogs diagnosed with CPSS, is currently under consideration for its potential to induce an intestinal microbial imbalance. In a recent investigation (Squire et al., 2022 [145]), attempts were made to establish a correlation between the faecal microbiome and dogs under medical management for a definitive CPSS diagnosis. Given metronidazole is often used to help mitigate the secondary clinical manifestations arising from CPSS, it is vital to recognize any impact of these antimicrobials on overall dysbiosis. Consideration and assessment of these interactions is vital in building a deeper understanding of therapeutic outcomes for these patients. This prospective cohort study enrolled 27 client-owned dogs in taking faecal samples and collecting follow-up data. The study assessed the faecal DI and the abundance of individual bacterial species using real-time qPCR. Alongside these assessments, data related to medical management, client reactions, clinical parameters and outcomes were compiled. Logistic regression analysis was employed in examining the potential association between all the collected data. Surprisingly,

no significant association was found between the dogs receiving metronidazole and their DI values. However, even in the absence of a discernible correlation with the DI, this study found a notable connection between metronidazole administration and an increased abundance of E. coli. This particular finding aligns with a previously mentioned metronidazole trial, undertaken by Pilla et al. (2020 [118]) involving healthy canine subjects.

TYLOSIN

Tylosin, is a solely veterinary registered antibiotic, that takes bacteriostatic action in mostly anaerobic gram + bacteria, some gram – bacteria and mycoplasma spp. (Stone et al., 2009 [146]; Liu & Douthwaite, 2002 [96]). This macrolide classed antibiotic binds to the 50S subunit of the bacterial ribosome, preventing protein synthesis by preventing polypeptide chains from forming, hence eliciting an antibacterial effect (Arsic et al., 2017 [12]). Additionally, tylosin has been postulated to reduce inflammation through affecting the synthesis of multiple inflammatory mediators and cytokines (Cao et al., 2006 [26]). Therapeutically, it has good effect on relevant gastrointestinal pathogens such as Cl. perfringens and Campylobacter spp, which are both implicated in the etiopathogenesis of chronic and intermittent diarrhoea in dogs (Marks & Kather, 2003 [101]). Furthermore, tylosin is commonly employed to address such conditions as SIBO or chronic enteropathy (Suchodolski & Steiner, 2003 [154]). It has been a widely recommended treatment for chronic enteropathies in dogs but remains unclear if therapeutic dosages solely target intestinal pathogens or also induce changes to general intestinal flora in dogs with diarrhoea (Suchodolski et al., 2009 [153]; Pinna et al., 2020 [122]). The exact mechanism behind the improvement of faecal consistency remains uncertain, however it is believed to be associated with modulation of intestinal microbiota (Manchester et al., 2019 [100]). Previously, researchers hypothesized that dogs with tylosin-responding diarrhoea may share a common tylosin-susceptible enteropathogen (Westermarck et al., 2005 [175]; Kilpinen et al., 2011 [81]). Nonetheless, this has not been validated through empirical evidence. Alternative theories regarding the mode of action behind tylosin effect, encompass the promotion of beneficial commensal bacteria, a reduction in small intestinal bacteria load, and perhaps the

inhibition of various mucosal immune responses (Menozzi et al., 2005 [104]; Cao et al., 2006 [26]). The following studies will discuss tylosin use and its scientifically deducted microbial effects.

The application of tylosin does not offer a definitive resolution of ARE, as diarrhoea frequently returns post discontinuing the therapy (Kilpinen et al., 2014 [82]). As a result, a considerable number of dogs necessitate lifelong treatment to achieve disease control (Westermarck et al., 2005 [175]). Among antibiotic solutions, there is a consensus that tylosin is a highly effective therapeutic choice. In light of this, the term 'tylosin-responsive diarrhoea' has been coined to underscore the drug's efficiency in managing dogs afflicted by IID (Westermarck et al., 2005 [175]). This is why research in this area is crucial, as better understanding of why certain dogs improve with tylosin may help towards more lasting treatments.

Marclay et al. (2022) conducted a randomized, placebo-controlled study explore the impact of tylosin therapy on 16 healthy dogs with or without faecal microbial transplantation (FMT), on the recovery of the faecal microbiome. Antibiotics are known to cause gut dysbiosis and deregulate bile acid bio-transformative reactions in dogs, despite tylosin being frequently utilized as a treatment for digestive issues and imbued intestinal dysbiosis (German et al., 2010 [53]; Volkmann et al., 2017 [166]). All dogs involved were administered oral tylosin 20 mg/kg daily for a week. Through days 8-21, the dogs were divided into three groups to receive either: 1) a single enema FMT 2) daily oral FMT capsules 3) daily placebo capsules. Faecal samples were collected at regular intervals, up until day 42 for analysis, where qPCR assessed the abundance of bacteria taxa and consequentially the dysbiosis index. Additionally, GC-MS was employed to measure the faecal concentrations of unconjugated bile acids (UBA). Results showed tylosin having a notable impact on the faecal microbiota and BA concentrations by day 7 in all dogs studied, but restoration to normal baseline values was rapid post tylosin was discontinued. It had been hypothesized that FMT would hasten recovery of the antibiotic-induced dysbiosis, but this was not the case. Therefore, further investigation is warranted to establish the efficiency of FMT in the treatment of antibiotic

related dysbiosis. As all participating dogs in the experiment were deemed healthy ahead of the trial, it would be of considerable interest to investigate if dogs with diagnosed unbalanced biomes would exhibit any influence on the outcome.

A similar prospective, randomized controlled clinical trial was undertaken to evaluate over time, the impact of tylosin on the intestinal microbiome and UBA concentration (Manchester et al., 2019 [100]). The faecal microbiota was examined through daily faecal scoring and faecal sampling (Days 0,7,21 and 63). 16 healthy dogs were randomly assigned into a placebo or tylosin group, where in depth faecal microbial testing was conducted using qPCR and 16S rRNA sequencing and UBA were analysed via GC-MS methodology. The results indicated that faecal scores remained unchanged in both groups, however tylosin treated dogs exhibited decreased bacterial species diversity, which is a common finding in dogs with a chronic enteropathy (Minamoto et al., 2015 [106]; Minamoto et al., 2019 [105]). Specifically, anaerobic species Fusobacteriaceae and Veillonellaceae were notably decreased. Veillonellaceae is notable as this taxon is an essential SCFA-producing core bacteria (Minamoto et al., 2019 [105]), and reduced availability of SCFA equates to a lesser amount of the primary food source of colonocytes (Minamoto et al., 2019 [105]). Moreover, primary UBA concentrations in the dogs receiving tylosin were found to be increased at day 21 and day 63. Contrary to the previous study, recovery rates of intestinal microbes were found to vary after the discontinuation of tylosin, suggesting a more persisting effect. However, tylosin application was proven to again induce faecal dysbiosis in otherwise healthy dogs. This has clinical importance as it highlights the potential gut microbial and BA impact in dogs when treated with a tylosin antibiotic. This warrants further attention and consideration when applied in veterinary practice.

As noted in previous discussions, ARE has undergone reclassification within the framework of chronic enteropathy, and now falls under the IID category. A multicentric prospective study performed by Bottero et al. (2022 [25]) endeavoured to elucidate the effect of tylosin on the gut microbiota of dogs with suspected ARE, and their overall clinical progression over a period of 120 days. The study encompassed 30 dogs, with group A comprising of 15 dogs

afflicted with chronic diarrhoea, and group B consisting of 15 dogs serving as a control. Initially baseline evaluation of species diversity between the 2 groups was significantly different, with group A exhibiting considerably reduced richness of Lactobacillaceae. After a period of 30 days, 9 dogs in group A experienced a relapse of diarrhoea, and a notable disparity in alpha-diversity was detected. The study's overall conclusion revealed that whilst tylosin is known to influence the intestinal microbiota of dogs with ARE, no specific characteristics were detected in the microbiota of dogs belonging to group A. Hence no typical microbiota profile was discovered that could clinically aid in the clinical identification of ARE or chronic diarrhoea. These findings align with the findings of a previous study (Suchodolski, 2016 [150]) where no repeatable dysbiotic patterns were concluded. To enhance clinical understanding, additional in-depth research is required to investigate the precise microbial changes underlie dysbiosis and enteropathies.

Pinna et al. (2020 [122]) conducted a noteworthy study on tylosin, which not only examined the impacted faecal microbiota, but also explored how tylosin treatment affects their metabolism. Contrary to aforementioned tylosin studies, this experiment adopted an atypical approach by employing in vitro methodologies, utilizing faecal microbiota from healthy dogs. They also trialled prebiotic oligosaccharides like fructooligosaccharides, galactooligosaccharides or xylooligosaccharides, to see if this had any counter-effect. Tylosin treated samples were found to have a higher pH, increased Clostridium cluster I and a lesser concentration of Lactobacilli. Notably, the combination of tylosin with prebiotics demonstrated a counteractive reaction on certain negative effects of tylosin. For instance, the prebiotics were shown to preserve beneficial commensal bacteria populations such as lactobacilli and clostridium cluster XIVa. They also showed a VFA protective effect, which are vital microbial fermentative end-products recognized for their significance in supporting enterocytes. The sample analysis method relied on qPCR analysis and did not explore as many specific bacterial groups as previously discussed. As a result, care must be taken when interpreting the results and trying to draw parallels. However, it is intriguing to note that prebiotics exhibited a mitigating effect on how tylosin affects microbial diversity.

DIETARY EFFECTS

Veterinary dietary intervention is believed to exert modulatory effects on the intestinal gut microbiome, making it a common treatment choice in dogs afflicted with food-responsive chronic enteropathy (Bresciani et al., 2018). Carefully managed elimination diets can be used to gain vital information on CE at an individual level, which can help towards diagnosis in cases that respond favourably. The primary objective is to deliver complete, well-balanced nutrition whilst circumventing any potential reactive substances and dysbiosis resolution (Allenspach et al., 2007 [7]; Gaschen & Merchant, 2011 [50]; Dandrieux, 2016 [33]). The overarching goal is to mitigate GI mucosa irritation, sustain normal GI motility and ultimately ameliorate or at least diminish presenting clinical signs (Purina Institute, 2021 [124]). Many CE sufferers respond positively to controlled nutritional therapy, negating the need to resort to furthermore invasive tests like biopsies and histopathological tests (Wernimont et al., 2020 [174]). Elimination diet trials are now frequently prescribed as a preliminary step to endoscopy in dogs exhibiting mild to moderate GI signs, when no other underlying causes are evident (Walker et al., 2014 [169]; Allenspach et al., 2016 [4]).

Historically, as natural carnivores, dogs have held a preference for high protein diet (Macdonald & Rodgers., 1984 [97]; Clauss et al., 2010 [29]). However, contemporary domestic dogs mainly consume diets high in carbohydtrates as live in urban environments as pet companions. This dietary lifestyle exposes them to similar health challenges as human beings, such as obesity, metabolic diseases and cancer (Kieler et al., 2017 [80]; Bermudez Sanchez et al., 2020 [21]; Pilla & Suchodolski, 2021 [121]). Overall health of dogs is massively impacted by the composition and activity of microbiota, and this has been linked to the development of various diseases (Lee & Hase., 2014 [86]). Thus, gaining a more profound understanding of the microbiota in pets and its influencing factors, holds massive clinical significance in fostering canine health.

The majority of microbiome research conducted on dogs has focused on investigating the clinical effects of extruded diets, which comprise 95% of commercially available canine diets

(Pilla & Suchodolski, 2021 [121]). Many different formulations exist, but largely include a high carbohydrate load, to limit potentially reactive animal proteins. Although still in the minority, there has been a recent increase in the number of owners choosing homemade, raw and frozen prepared diets, which tend to be more meat and protein forward. (Freeman et al., 2013 [49]; Davies et al., 2019 [35]). More research is needed into the role these more modern dietary regimes have in canine dysbiosis and CE.

In 2018, Bresciani et al. conducted a study that offered valuable insight into the faecal microbiota alterations resulting from the provision of animal-protein free diet to canine sufferers of CE. The findings of this study showed a marked improvement in faecal microbe richness in dogs with CE, following the dietary intervention, but no significant microbial changes. The faecal microbiota of the healthy treated dogs exhibited relatively unremarkable results. This research clearly demonstrates the link between dysbiosis and CE, whilst also positively highlighting a dietary solution. Despite the strong links shown, the 60-day trial was relatively small and heavily relied on the owner for sample collection, handling and storage. The involvement of particular breeds, home environments and even routine may introduce a potential bias. It would be of considerable interest to observe additional studies to further explore and expand upon these findings.

Unlike the majority of chronic enteropathy research trials that focus on fully matured dogs, Vuori et al. (2023 [167]) carried out a significant cross-sectional, longitudinal epidemiological study on puppies, monitoring their development into adulthood. Their investigation aimed to elucidate the potential impact of early diet on future occurrence of inflammatory gastrointestinal disorders later in life. Current knowledge projects that the gut microenvironment and host immune system play a major role in the development of inflammatory intestinal disturbances, although the exact etiology of chronic enteropathy remains elusive (Tizard & Jones., 2018 [162]; Harris & Chang, 2018 [60]). Principal component analysis and logistic regression were employed to analyse data from a food frequency questionnaire collected from Finnish companion dogs, encompassing a total of 16,607 answers. The study revealed that certain feeding practices during puppyhood (2-6

months old) and adolescence (6-18 months old) had a protective effect against developing chronic enteropathy in later life. Specifically, feeding a non-processed meat-based diet and allowing the dog occasional suitable human meal leftovers in developmental years, were associated with a reduced risk of developing chronic enteropathy. Notably, the inclusion of raw bones and cartilage and berries were also associated with a lower chronic enteropathy incidence. Conversely, certain feeding practices were identified as significant risk factors for chronic enteropathy occurrence later in life. These included feeding younger dogs an ultra-processed, carbohydrate-based diet such as dry dog food kibble and also the inclusion of raw-hide snacks. This study highlights the impact of diet and the potential role it plays on intestinal health. However, more in-depth studies, analysing the precise microbial effects and exact frequencies of specific dietary interventions are warranted to promote its findings.

Another non-animal protein dietary trial (Kerr et al., 2013) demonstrated the impact of incorporating 25% navy beans (also known as haricot beans) on the canine microbiome over 4 weeks. Similarly, to the previously discussed study, the prescribed diet had no major effect on the intestinal microbiome, but after 4 weeks on either the bean/control diet, an increase of Phylum Firmicutes was observed, along with decreased levels of Phyla Actinobacteria and Fusobacteria when compared to the baseline levels. Numerous studies have supported the beneficial effects of bean consumption in human and rat models, towards combating chronic inflammatory diseases (Sánchez-Tapia et al., 2020 [134]; John et al., 2023 [78]; Mirmohammadali & Rosenkranz, 2023 [107]). The application of more bean-involved dietary studies to dogs suffering from dysbiosis or chronic enteropathy would perhaps be valuable.

A surprising link has been discovered between the intestinal microbiome and obesity (Kieler et al., 2017 [80]). In this prospective, non-randomized 12-week trial, 18 healthy medium-large breed dogs were examined, and fed Royal Canin's Satiety Support restrictive diet. This dry feed contains high protein, fibre and low fat levels, and was combined with a sedentary lifestyle as per the owner, to investigate the interplay between low exercise, weight loss and the gut microbiota. An additional subset of 8 dogs were enrolled in an exercise program in

addition to the dietary intervention. Faecal samples were collected and their weights recorded periodically. It was concluded that exercise did not alter total weight loss, food allowance or faecal microbiota composition. However, the abundance of Megamonas negatively correlated with weight loss rate. Dogs found to lose weight more readily exhibited a lower Ruminococcaceae abundance compared to those dogs with a slower weight loss. Both of these microbial changes, suggest that gut microbial composition favouring SCFA production, may hinder weight loss rates in dogs. Minamoto et al. (2019 [105]) found dogs with chronic enteropathy to also have decrease SCFA concentrations, and so more research into these microbial metabolites is warranted.

Several studies have investigated the effect of meat-based raw diets on the gut microbiome of heathy dogs, which will not necessarily lend data to dysbiosis sufferers. However due to growing popularity in developed countries and its microbiome effects, it requires brief discussion (Dinallo et al., 2017 [40]; Davies et al., 2019 [35]). Short-term consumption of a diet with significant macronutrient differences, such as an omnivore to carnivore transition, have been proven in humans to rapidly alter gut microbiota profiles (David et al., 2014). Contrary to typical control diets, raw diets are characterized by high protein and low carbohydrate content, with limited fibre.

Schmidt et al. (2018 [137]) explored the effect of feeding 27 dogs a BARF diet and 19 dogs a commercially prepared diet, upon the faecal microbiome. Naturally passed faecal samples were analysed for differences in crude protein, fat, fibre and nitrogen-free extract, and the faecal microbiota was analysed by both 16S rRNA gene sequencing and qPCR assays. A further untargeted faecal metabolome approach was applied to 10 BARF dogs and 9 commercially-fed studied dogs. Dogs in the BARF group received a diet notably higher in protein and fat, whilst their intake of nitrogen-free extract and fiber was significantly lower than the commercially-fed group. Through linear discriminat Analysis Effect Size (LefSe), higher abundances of lactobacillales, Enterobacteriaceae, Fusobacterium and Clostridium were identified in the BARF group. Whilst commercially-fed dogs exhibited higher abundances of Clostridiaceae, Erysipelotrichaceae, Ruminococcaceae and Lachnospiraceae,

Negatively, BARF fed dogs were revealed to have notably higher amounts of E.coli and C. perfringens, along with an increased dysbiosis index. No disparities were observed in faecal BA concentrations, but the BARF group exhibited higher faecal cholesterol concentrations compared to the conventionally-fed dogs. The study concluded before any long-term impact of the differing diets could be assessed, therefore it is not known whether the changes deducted from these results will incur future intestinal disease. However, the study did find intestinal microbial changes (Suchodolski, 2012) frequently observed in GI diseases, in the BARF group.

The incorporation of dietary fibre into canine diets has been documented as an efficacious regulator of the gastrointestinal microbiota (Wernimont et al., 2020 [174]; Pilla & Suchodolski, 2021 [121]). Resistant starch (RS), a fermentable dietary fibre, has garnered considerable attention, particularly within the realm of human nutritional medicine, due to its ability to positively influence the gastrointestinal tract and its associated microbiota. (Walsh et al., 2022 [170]). This influence is primarily achieved by its ability to stimulate heightened butyrate production from SCFA fermentation (DeMartino & Cockburn, 2020 [36]). Mackei at al. (2022 [99]) conducted a large comparative study on 30 beagles that corroborates the use of fibre in dogs for this purpose. Over a 15-day trial duration, all dogs received the same standardized diet. On top of this, half the cohort were supplemented with lactulose whilst the other half received a psyllium supplement. Both lactulose and psyllium were shown to alter the intestinal VFA, demonstrating noteworthy increases through GC-MS analysis of faecal samples. However, elevated n-butyrate concentrations were only observed in the psyllium-fed cohort, suggesting that prebiotic application may promote further effects on the canine hindgut. These findings hold large significance in the context of managing patients with intestinal disorders and portosystemic shunts (PSS), offering perhaps a future therapeutic avenue. Future studies exploring psyllium and the intestinal microbiome, could pursue a more breed-diverse study group. This may serve as better representation for canine animals as a whole and help towards reducing bias.

Fundamentally, these varieties of starch possess the capacity to resist small intestinal breakdown and evade digestion, which allows for colonic fermentation by specialized microbiota (Li et al., 2018 [93]). This process indirectly modulates the environment of the colon, promoting healthy colonocytes, SCFA-producing bacteria, fermentation activity and the reduction of faecal pH (Birkett et al., 1996 [22]; Peixoto et al., 2017 [117]). Functioning as a histone deacetylase inhibitor, Butyrate has been demonstrated to operate in an epigenetic capacity in hindering the proliferation of cancerous colorectal cells engaged in the Warburg effect, and also in reducing gut inflammatory markers (Donohoe at al., 2012 [42]; Donohoe et al., 2014 [43]). Although RS has demonstrated exceptional efficacy in augmenting butyrate levels on a human population scale, it is evident that uniform benefits do not extend to every individual, with some participants even seemingly unresponsive to RS (Knudsen et al., 2018 [83]). This observation underscores the significant influence exerted by an individual microbiome, and necessitates a more comprehensive exploration into RS and its effects. Also supporting the need for additional investigations, is the incredibly limited scope of research exploring the impact of RS on canine microbiota. Despite promising studies in rodents and pigs (Rodríguez-Cabezas et al., 2010 [130]; Tan et al., 2021 [159]), canine application seems to be in short supply, perhaps related to canine intestinal anatomical differences.

Beloshapka et al. (2021 [18]) investigated the clinical impact of adding varying levels of resistant starch to the diet of healthy dogs, using an incomplete latin-square study design and five randomly assigned 21-day experimental periods. Each dog acted as its own control, as faecal samples were only collected after day 18, to allow for a dietary adjustment period. This study aimed to systematically examine the total tract macronutrient digestibility, fermentative end-product production and composition of faecal microbial populations of 7 dogs, after being fed increasing concentrations of fermentable resistant corn starch. Statistical analysis involved assessing linear and quadratic effects using a Statistical Analysis System. The experiment revealed that an increased RS consumption led to linear reductions ($P < 0.05$) in digestibility across the board and in faecal pH. Additionally, faecal output, Lactobacillus, Prevotella, Blautia and Faecalibacterium spp. exhibited linear increase ($P < 0.05$). The statistical changes in microbiota were minimal, but the slight shift infers potential importance

of further exploration in dogs with much larger sample sizes. Beloshapka et al. (2021 [18]) proposed a potential link between amplified microbial activity and augmented faecal nitrogen excretion in healthy dogs, however further application of such resistant starch supplements in clinical population studies may be insightful.
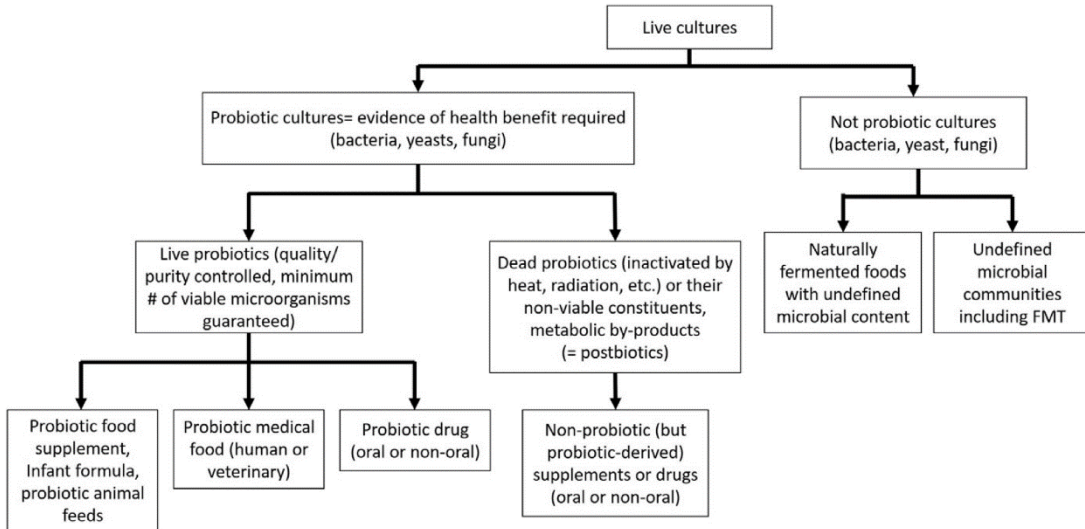
PROBIOTICS

The International Scientific Association for Probiotics and Prebiotics have termed probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit to the host" (Hill et al., 2014 [63]). The ISAPP non-profit organization was established in 2002 to champion the flow of accurate scientific information regarding probiotics and their use. Numerous instances of mislabelling and misbranding have been documented commercially (Weese, 2002 [171]; Weese & Martin, 2011 [172]; de Simone, 2019 [37]) which the ISAPP aims to clarify this in Fig.7. by separating probiotics into classes and defining clearly the scope and appropriate use. Largely lactic acid bacteria (LAB) are applied in animals as their numbers tend to be lower in chronic enteropathy sufferers, but probiotic therapy is also commonly utilized in human medicine (Sarowska et al., 2013 [135]; Rallis et al., 2016 [126]). Other important canine strains include: Bifidobacterium, Enterococcus faecium and Saccharomyces boulardii yeast (Dandrieux & Mansfield, 2019 [34]). Different classes of probiotics exert their effect through various mechanisms: nutrient competition, antibacterial effect and pathogen adhesion (Sarowska et al., 2013 [135]). By fostering the growth of beneficial bacteria within the GI tract, probiotics facillitate the restoration of balanced intestinal microbiota, thereby enhancing digestive function. They also lend prospective advantage by positively modulating the immune system, mitigating systemic stress and susceptibility to infection. Additionally, probiotics are thought to play a role in managing allergies, enhancing growth and development and even obesity management (Abenavoli et al., 2019 [1]).

Research examining probiotic use in dogs with CE or other forms of intestinal dysbiosis tend to be bench-top or clinical trials, where different probiotics treat different breeds, suffering

from various intestinal issues. Therefore, drawing any definitive conclusions from such studies or making parallels is incredibly difficult.

Fig 7. ISAPP Probiotic framework (Hill et al., 2014 [63])

Sauter et al. (2006 [136]) conducted an interested albeit multifaceted study on 21 client-owned dogs with FRE, assessing the potential beneficial effects of probiotic supplementation on intestinal cytokine patterns and intestinal microbiota. During endoscopy, intestinal tissue samples and faecal specimens were collected and trialled against a probiotic placebo. Through mRNA technique the abundance of interleukins, TNF-α, TGF-ß1 and IFN-γ were analysed, and lactobaccilus spp., bifidobacterium spp., enterococcus spp., enterobacteriaceae quantified. The probiotics supplemented were evaluated through intestinal faecal sampling. All dogs supplemented with probiotics in this double-blinded study showed a significant reduction in CIBDAI with lactobacillus spp. numbers increasing during the treatment phase. Post probiotic treatment, over 60% of the dogs had at least one probiotic strain identified in their flora, suggesting probiotics had a persisting effect. Even the placebo group received an elimination dietary intervention and clinically improved, so it cannot be stated that probiotic prescription alone had significant effect. To understand the full benefit of probiotic treatment, more standardized stand alone trials are warranted.

In cases of IBD, dogs are sometimes prescribed probiotics, in conjunction with standard immunosuppressive therapy. White et al. (2017 [176]) conducted a randomized study where dogs diagnosed with IBD received either standard therapy alone, or in combination with probiotic supplementation. Both treatment regimens demonstrated comparable modulation of the number of mucosal bacteria found in the dogs, leading to increased bacterial numbers found in the adherent mucosal layer. Additionally, both treatments were associated with a swift clinical recovery, even though no physical reduction of histopathological inflammation was identified. Curiously, It was of particular interest that only the group receiving the supplemented probiotics, were found to exhibit enhanced expression of tight junction proteins. This implies that despite lacking colonization, probiotics may positively exert an effect on mucosal homeostasis.

In another study by Rossi et al (2014 [132]) dogs afflicted with IBD, a multi-strain probiotic exhibited successful results as an alternative to the combination therapeutic protocol of prednisolone and metronidazole. Demonstrated in the study was the utilization of a combined therapy administered over a span of 60 days, succeeded by a 30-day interval dedicated to the elimination of residual effects. Both treatment groups experienced a significant decrease in clinical assessment scores over time, although the treatment group receiving probiotics showed a slower remission of clinical signs. However, upon evaluating specific bacterial taxa within the gut microbiome via qPCR, only the probiotic group was found to have an increase in the abundance of Faecalibacterium spp., a strain not included in the probiotic mix applied. This is an important butyrate-producing strain, a SCFA that is undisputed in maintaining intestinal health (Rivera-Chavez et al., 2016 [128]). This study interestingly, did not show metronidazole to significantly alter bacterial proportions of Bacteriodetes, Firmicutes, Fusobacteria, Bifidobacterium, Lactobacillus, Faecalibacterium, Escherichia coli and Clostridia perfringens. This potentially could be attributed to variables such as the health status of the sampled dogs, the implementation of a wash-out period and dissimilarities in methodologies employed.

An alternative perspective delves into the infective origins of canine intestinal dysbiosis, as recent research has drawn conclusions regarding the correlation between parvovirus and substantial disruption to the composition of the intestinal microbiota (Park et al., 2019 [115]). Comparative analysis in this study, revealed a notable disruption in alpha diversity, as well as decreased richness of species. In conjunction with this, Arslan et al. (2012 [13]) performed a study that aimed to assess the potential benefit of using probiotic therapy in comparison to standard supportive therapy alone. This study encompassed a cohort of 20 mixed-breed puppies aged between 1-6 months. All involved puppies were identified as 'naturally infected' based on presenting clinical manifestations of enteritis and a definitive positive result from a commercially available ELISA test. These puppies were evenly divided into two equivalent groups, with both groups receiving supportive symptomatic treatment. However, group 1 also received a multi-strain oral probiotic supplement. Over the course of the trial, it was observed that seven dogs in group 1 (70%) and nine dogs in group 2 (90%) successfully survived the infection. Furthermore, puppies in group 2 exhibited a notably expedited recovery in terms of clinical scores, when compared to their counterparts in group 1, who did not receive any probiotic treatment. Analysis of blood parameters also proved probiotic-supplemented puppies to demonstrate significant improvement in leukocyte and lymphocyte counts during the treatment period. These findings suggest that the administration of probiotics may yield benefits in the treatment of canine parvovirus, potentially reducing the duration of recovery and even mitigating the associated mortality rate linked to this infection. Conducting more research to substantiate any relationship between canine parvovirus and intestinal dysbiosis would hold clinical significance, and potentially facilitate more efficacious treatment approaches in the future.

DISCUSSION

This literature review has aimed to offer a comprehensive and thorough overview of the current research pertaining to the use of metronidazole, tylosin, dietary intervention and probiotics in the management of intestinal dysbiosis and related conditions in dogs. Current studies have examined these methods as stand-alone therapy treatments but also highlighted the complex interplay of a poly-treatment approach on the gut microbiome and clinical outcomes. Whilst these furnished valuable insights into potential benefits and associated intricacies, it has also emphasized the need for more rigorous and standardized trials to fully explore the effects of such prescriptions.

Many studies have explored the repercussions of metronidazole on the microbiome and its bearing on therapeutic outcomes in dogs. The study by Pilla et al. (2020 [118]) delved into the ramifications of a 14-day metronidazole regimen. The outcomes unveiled not only a decline in microbial diversity but corroborated the plausibility of prolonged metronidazole impact on the microbiome. In contrast, the preceding study of healthy dogs by Igarashi et al. (2014 [70]) eluded that the influence of metronidazole on the gut microbiota is merely transitory. This discrepancy in findings, underscores the imperative for further investigation aimed at comprehensively exploring the clinical and microbial ramifications of metronidazole therapy.

An additional inquiry conducted by Rudinsky et al. (2022 [133]) pursued an examination of metronidazole therapy in dogs afflicted with non-infectious acute colitis. The study uncovered that metronidazole engendered an adverse effect on the DI but interestingly extended the remission duration of the treated dogs. This amplifies the intricate dimensions of the influence, metronidazole holds on gut health, and potentially rationalizes why it was such a commonly prescribed medication for gastrointestinal ailments in the past. Despite this promising finding, navigating the potential downfalls of the therapy is vital, especially in dogs already suffering some form of dysbiosis. Hence, a more extensive understanding of metronidazole and the exact biological reactions it incurs is warranted.

While discernible shifts in the intestinal microbiome consequent to metronidazole have surfaced, the precise timeline, implication and affected bacterial species are yet to be definitively studied. Many studies discussed during the course of this paper, concluded slightly differing results. The investigations spotlighted in the discourse, emphasize the the need for more extensive, longitudinal research to better understand the potential effects of metronidazole therapy. Variation in approach to this issue, could explain why research has drawn such wide, assorted conclusions.

Marclay et al. (2022) conducted a controlled study exploring the impact of tylosin therapy on the recovery of the faecal microbiome of healthy dogs. Despite being employed to treat digestive disorders and intestinal dysbiosis, the potential of tylosin to induce gut dysbiosis and disrupt BA bio-transformative reactions is recognized This study found tylosin to have a rapid impact on the faecal microbiota and BA concentrations by day 7, although restoration to baseline values occurred swiftly after discontinuation. The effects of tylosin therapy were deemed temporary, but as the study focused on microbiota modulations in healthy dogs, investigating its influence on dogs with existing biome imbalances could yield some further valuable insight. Interestingly, Manchester et al. (2019 [100]) also conducted research on the impact of tylosin on healthy dogs, and found opposing conclusions, that implied more persistent effects of tylosin therapy. Bottero et al. (2022 [25]) conversely examined the effect of tylosin on dogs suffering with chronic enteropathy and demonstrated notable variations in alpha microbial diversity in 30 days. Hence similar to the earlier statements made regarding the metronidazole research discussed, more comprehensive examination of this antibacterial drug is also required.

A completely unique approach was applied by Pinna et al. (2020 [122]) who investigated the effects of tylosin on faecal microbiota and metabolism through in vitro methods. Tylosin treated samples were found to be of higher pH, increased Clostridium cluster I and decreased Lactobacilli. Prebiotics were also shown to mitigate some of the adverse effects of tylosin, preserve beneficial bacterial populations and support the production of fermentative end-products. Although this study utilized a different analysis method and did not explore specific

bacterial groups extensively, the interaction between tylosin was valuable and the effect of the prebiotic intriguing. In summary these antibacterial studies collectively contribute to the understanding of the intricate impact tylosin exerts on canine gastrointestinal health. They highlight the potential for intestinal dysbiosis, microbial alteration and bile acid disruption induced by tylosin therapy administration. The diverse findings underscore the complexity of effects and possible interactions with various treatments, necessitating further research to decipher the precise implications of their use as safe future therapies.

When investigating the importance of dietary influence on gut microbiota and disease in dogs, the impact of various dietary modulations highlighted the positive role nutrition plays in managing gastrointestinal health. Many dietary treatment avenues were discussed in this paper, providing insight not only into effects on the microbiota, but also other canine afflictions such as chronic enteropathy and even obesity (Kieler et al., 2017 [80]; Vuori et al., 2023 [167]).

Strategically devised elimination diets continue to serve as a pivotal diagnostic tool at the individual level, however this paper discussed various other dietary approaches such as non-animal protein, BARF and resistant starch (Kerr et al., 2013; Schmidt et al., 2018 [137]; Beloshapka et al., 2021 [18]). Each of these studies offered a valuable perspective on the potential impacts of specific prescribed diets on faecal microbiota. Nevertheless, the absence of significant findings underscores the necessity for further research into this domain.

In particular, the work Vuori et al. (2023 [167]) demonstrated the link between early dietary choices and the development of chronic enteropathy later in life, proving the long-term implications of diet on canine gut health. This highlights the pivotal role veterinary professionals hold in comprehending early dietary selections and their potential future health implications. More research into the maturation of the canine microbiome and the microbial consequences of early dietary choices are warranted, to empower the veterinary profession in providing informed counsel to puppy owners.

The incorporation of dietary fibre into canine diets has been proven to promote healthy gastrointestinal microbiota (Wernimont et al., 2020 [174]; Pilla & Suchodolski, 2021 [121]; Mackei et al., 2022 [99]). Mackei et al. (2022 [99]) conducted an extensive study into the application of dietary fibre in beagles. Through gas-spectrum chromatography (GC-MS) analysis, psyllium was was observed to an increase in intestinal volatile fatty acid (VFA) content within collected faecal samples. Dogs recieving psyllium supplementation demonstrated notably higher concentrations of n-butyrate, indicating the potential benefits this prebiotic exerts on hindgut fermentation. While broader research involving a more diverse range of dog breeds would enhance the robustness of these findings, this study holds significant promise for the treatment of various intestinal disorders.

A fermentable dietary fibre of particular note, Resistant Starch (RS) has shown promise in human trials (Walsh et al., 2022 [170]), however current canine research supporting any positive effect is limited. Beloshapka et al. (2021 [18]) found increasing RS intake in a healthy dog population to reduce digestibility and faecal pH. The study also revealed minor increases in faecal microbial populations and output, indicating that RS may indeed influence the canine intestinal microbiota. This underscores the importance of additional research into RS supplementation in for dogs. To delve deeper into potential associations, more extensive trials involving RS application in dogs are necessary. These trials should involve larger study cohorts and potentially include dogs with more disrupted microbiomes, to provide valuable insight.

However, elevated n-butyrate concentrations were only observed in the psyllium-fed cohort, suggesting that prebiotic application may promote further effects on the canine hindgut. These findings hold large significance in the context of managing patients with intestinal disorders and portosystemic shunts (PSS), offering perhaps a future therapeutic avenue. Future studies exploring psyllium and the intestinal microbiome, could pursue a more breed-diverse study group. This may serve as better representation for canine animals as a whole and help towards reducing bias.

Probiotics have emerged as a very promising therapy avenue for managing GI dysbiosis in dogs, as indicated by studies eliciting potential benefits in not only modulating the gut microbiota, but also in facilitating clinical recovery. However, the intricate, multifaceted nature of the gut microbiota, coupled with the variability of available probiotic strains, make it essential to conduct further research to ascertain optimal strains, clinical dosages and appropriate treatment durations. The studies discussed contribute valuable insights into the potential advantages of probiotic supplementation in the context of canine gastrointestinal health, both in non-infectious and infectious cases. All discussed probiotic studies were united in demonstrating that probiotic treatment was associated with a reduction of presenting clinical scores

In regards dogs afflicted with enteropathy disease (Sauter et al., 2006 [136]; Rossi et al., 2014 [132]) where a link to intestinal dysbiosis is well-established, probiotics were found to lead to an increased abundance of specific intestinal bacteria. Particularly of note, Rossi et al. (2014 [132]) where probiotic supplementation was observed to increase the presence of beneficial butyrate-producing strain of bacteria in the gut. This implies a potential advantage of probiotics in enhancing the bacterial composition of the gut microbiome, and therefore highlighting their potential merit in treating intestinal dysbiosis.

White et al. (2017 [176]) further expanded on the benefits of probiotics with their randomized study, revealing that only the probiotic-supplemented group displayed enhanced expression of tight junction proteins in the gut. This indicates a potential physically positive impact of probiotic use on gastrointestinal mucosal homeostasis.

While microbiome research focusing on the infective causes of canine dysbiosis appears to be less abundant, it should not be disregarded. Arslan et al. (2012 [13]) underscored the importance of considering intestinal dysbiosis, by demonstrating probiotic therapy to reduce recovery time and mortality rate in puppies suffering with canine parvovirus. While the study in question may be considered outdated, it is evident that the potential advantages of probiotic utilization may be very clinically valuable, and therefore warrant contemporary investigation

and consideration. Probiotics have been shown to hold significant potential as a valuable adjunctive therapy for a range of gastrointestinal canine conditions and so hold massive potential merit.

In summation, better comprehension of therapeutic modalities applicable to GI dysbiosis and in turn chronic enteropathy are necessary through more cohesive research. Present understanding is constrained and lacks the requisite standardization that is essential for deducting correlations and parallels. As modulations in the intestinal microbiome have been firmly established as being interconnected with general systemic health, acquiring additional knowledge is of paramount importance to enhance the efficiency of applied clinical interventions in veterinary medicine.

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THESIS PROGRESS REPORT

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Thesis progress report for veterinary students

Name of student: ...... Áine Sorcha O'Shea ....

Neptun code of the student: ... FAJAOH ....

Name and title of the supervisor: Prof. Ágnes Andrea Sterczer ....

Department: .. Internal Medicine ....

Thesis title: .. Canine Intestinal Dysbiosis : A Literature Review ....

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Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2022	11	10	Discussion of the topic	D Shz
2.	2022	12	05	literature snoppertion	D Shz
3.					
4.					
5.					

Grade achieved at the end of the first semester: ....

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
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1.	2023	02	08	Discussion of the structure	D Shz
2.	2023	05	14	Draft version	D Shz
3.	2023	09	12	Correction of the thesi	D Shz
4.	2023	10	05	Correction of the thei	D Shz

5.				

Grade achieved at the end of the second semester: ....5..........................

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

....
signature of the supervisor

Signature of the student: .....Áine O'Shea......

Signature of the secretary of the department: ...Czatala-Sóhr Renáta

Date of handing the thesis in.....17th Nov 2023...........

SUPERVISOR CONFIRMATION LETTER

I hereby confirm that I am familiar with the content of the thesis entitled

………………… Canine Intestinal Dysbiosis …………
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Date: Budapest, …13…day…11…..month …2023…year

DR. SZERDAER ÁGNES

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