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Study on gut bacteriome of canines with chronic enteropathies

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Abstract

Chronic enteropathy (CE) in canines [2] is a multifaceted gastrointestinal disorder that shares similarities with human inflammatory bowel disease (IBD) [1]. It is characterized by persistent inflammation of the intestinal mucosa, leading to symptoms such as chronic diarrhea, vomiting, weight loss, and abdominal discomfort. Despite advancements in veterinary medicine, CE remains a diagnostic and therapeutic challenge due to its complex pathogenesis, variable clinical presentations, and multifactorial etiology involving genetic predispositions, environmental factors, dietary influences, and dysbiosis of the gut microbiota.

This thesis investigates the pathogenesis, diagnostic approaches, and management strategies for CE in canines, with a particular emphasis on the role of the gut microbiome.
Utilizing next-generation sequencing (NGS) technology [52], fecal samples from 2 main projects [62], [63] were analyzed, Healthy and diseases with CE Yorkshire terrier, and healthy diseases with CE of mixed dog breeds.
Healthy dogs and those affected by CE were analyzed to identify microbial community shifts associated with disease states. The study included comparisons across breeds, including mixed breeds to examine breed-specific variations in microbiota composition.
With examination of Yorkshire Terriers CE, known as “Protein losing enteropathy (PLE)” [39]
Additionally, data from acute enteropathies project [58] was explored to identify potential links between acute and chronic conditions.
In this thesis we worked on understanding the microbial changes in both groups of CE.
The findings reveal significant alterations in the gut microbiota of CE-affected dogs, marked by a decrease in beneficial taxa such as *prevotella copri* and *Faecalibacterium prausnitzii*, and an increase in pathogenic bacteria including well known *Clostridium perfringens*, and new agents such as *Bacteroides* fragilis and *Ruminococcus gnavus*.
These shifts in microbial populations are associated with disruptions to intestinal barrier function, immune dysregulation, and chronic inflammation. [3], [5],

Therapeutic interventions, including dietary modifications, were shown to play a pivotal role in managing CE. [24], [25]
Stress management emerged as an equally important factor, particularly in working dogs

exposed to high-pressure environments, further highlighting the interplay between environmental stressors and intestinal health. [26]

This thesis aimed to contribute knowledge on CE by advancing our understanding of the disease's microbial underpinnings and offering insights into personalized gut health and therapeutic strategies in veterinary medicine. The identification of key microbial agents has diagnostic importance that paves the way for targeted that can develop in the future.

Absztrakt

The abstract will be translated into Hungarian, by courtesy of Professor Solymosi.

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1. Introduction

Inflammatory Bowel Disease (IBD) in humans [1], and Chronic enteropathy (CE) in dogs[2], are chronic gastrointestinal disorder characterized by persistent inflammation of the intestines, leading to a range of clinical symptoms that significantly affect the quality of life in affected humans and dogs.

While IBD is well-documented in humans, CE in dogs has garnered increasing attention from veterinary researchers due to its complex etiology, challenging diagnosis, and variable response to treatment.

In canines, CE presents with symptoms such as chronic hemorrhagic or non-hemorrhagic diarrhea, vomiting, weight loss, and abdominal discomfort, which can vary in severity.

The pathophysiology of CE in canines is multifactorial and remains incompletely understood, mirroring the complexities seen in human IBD.

The immune system plays a significant role, where an inappropriate immune response to intestinal antigens, including dietary components and the gut microbiota, leads to chronic inflammation [4]. Genetic factors are also implicated, with certain breeds showing a higher predisposition to developing CE, suggesting a heritable component [21].

Dysbiosis, or an imbalance in the gut microbial population, has been observed in dogs with CE [3], [5].

Moreover, environmental factors such as diet, stress, and exposure to pathogens such as *Giardia*[33] and *Parvovirus* [38] are thought to trigger or exacerbate the condition in genetically susceptible dogs.

The diagnosis of canine CE is challenging, takes long time and sources. For confirmation of CE disease, many methods are needed, such as: clinical evaluation, laboratory tests, imaging, and histopathological examination of intestinal biopsies taking by endoscopy. Despite advancements in veterinary diagnostics, this condition is often managed on a case-by-case basis, with treatments ranging from dietary modifications, restoration of gut microbiome and anti-inflammatory drugs to immunosuppressive therapies.

The treatment for CE these days is not unified, and sometimes considered as “a shot in the dark.” To control and manage this disease better, it is needed to find a better solution or mechanism that can target the disease completely.

In this thesis, we will focus on the current knowledge on the pathogenesis, diagnosis, and management of CE in canines, with a particular focus on the role of the gut microbiome. The gut microbiome plays a vital role in maintaining intestinal health by regulating immune responses, aiding digestion, and protecting against harmful pathogens. Imbalances in microbial communities, known as dysbiosis, contribute to gastrointestinal disorders like chronic enteropathy. Microbiota identification is commonly performed through techniques such as Next generation sequencing [52], with specific method for targeting the 16S rRNA gene, which assist in finding of specific bacterial markers, and shotgun metagenomics, which provides a broader view of microbial composition, enabling researchers to better understand the functional roles and diversity of gut bacteria. [55]

2. Literature review

2.1. Pathophysiology of Inflammatory bowel diseases (IBD) in humans, chronic enteropathies (CE) in canines and Protein losing enteropathies (PLE) in Yorkshire terriers.

2.1.1. Pathophysiology of the intestinal tract

Chronic enteropathies (CE) in dogs and Inflammatory bowel diseases (IBD) in humans are considered a persistent gastrointestinal (GI) disorder characterized by chronic inflammation of the intestinal mucosa with the following processes:

1. Disruption of Intestinal Barrier [1]:

The Epithelial layer of the intestine loses its function. The barrier of the intestine becomes prudent and increase of its permeability allows antigens to penetrate deeper layers, triggering an immune response.

2. Immune Dysregulation [2]:

Inappropriate activation of the gut-associated lymphoid tissue (GALT) occurs. Overreaction of immune cell components such as Th1, Th2, or Th17 cells, will lead to chronic inflammation.

3. Microbiota Dysbiosis [3]:

Dysbiosis of the gut microbiota composition will occur. A shift in the composition of the microbiome bacteria will be observed.
Bacteria that are considered “healthy” from the family of Bacteroides and Firmicutes, will decline significantly, while opportunistic pathogens such as E. coli, Clostridium spp. etc. will increase and will lead to inflammation and disturbance of the epithelial barriers of the intestine.

4. Chronic Inflammation:

Persistent immune activation will lead to infiltration of immune cells such as lymphocytes, plasma cells, and sometimes eosinophiles [4] into the intestinal mucosa. Cytokines such as IL-6, IL-8 and TNF-α, will cause increment of the intestinal permeability, in order to create inflammatory process.

5. Nutrient Malabsorption [5]:

The Inflammatory process damages the intestinal villi, reducing surface area for absorption. This will lead to malabsorption with weight loss, diarrhea, and malnutrition. In severe cases, it will lead to protein losing enteropathy (PLE), which will be discussed further on.

6. Structural Changes [3]:

Chronic inflammation can lead to fibrosis and to abrasion and destruction of the intestinal layers. It is possible to see with an endoscope, abnormalities such as: erosions, ulcers and destruction of the muscular layer, as seen in figure 1.

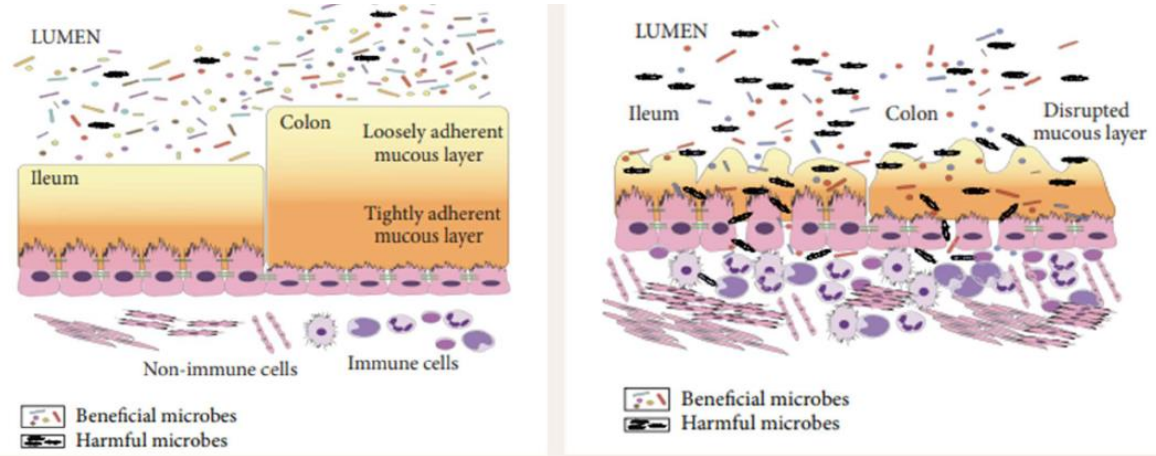

Figure 1: Structural changes of the intestine
[Scaldaferri et al., 2013[3]]

2.1.2. IBD in humans and its multifactorial causes

Inflammatory bowel diseases (IBD) refer to a chronic, persistent inflammation of the GI tract. including the stomach, small intestine, and colon. The exact cause of IBD is often unknown, but it is believed to involve a complex interplay of genetic, immune, and environmental factors. [6]

In humans, it is divided to 2 main diseases: [7]
(a. Crohn's disease (CD), which is characterized as inflammation through most of the GI tract, but mostly targets the small intestine and the cecal border between the small and large intestine.
(b. Ulcerative colitis (UC), which is characterized as inflammation throughout the large intestine.

The disease may result in clinical presentations such as: chronic vomiting, hemorrhagic or non-hemorrhagic diarrhea and weight loss. Those diseases are diagnosed histopathologically from intestinal biopsy taken with an endoscope. Biopsy results analyzed and can confirm an IBD disease by the type of inflammation in the lamina propria of the small and/or large intestine. The cause of IBD is multifactorial: from nutritional insufficiency [8], chronic stress [9], lifestyle management [10] and genetic factors [11].

The treatment for IBD disease can vary, from: corticosteroids to immunosuppressive medicines, depending on the severity of the disease and previous treatments.

Microbiome modification techniques such as:

1. **Antibiotics**: Overuse can reduce diversity, allowing opportunistic pathogens like *Clostridium difficile* to flourish, therefore treatment of antibiotics can help with balancing of the microbiome and decreasing *C.difficle* levels [12]
2. **Probiotics/Prebiotics**: can help with maintaining gut health, by increasing the amount of *Bacteroides* and *lactobacillus* bacteria [13], that are linked to microbiome health [14], [15].
3. **Fecal Microbiota Transplant (FMT)**: in this method, feces are compressed into a capsule, which the patient swallows. The capsule contains feces from an individual

with a healthy and normal microbiome [16].

it replaces dysbiotic bacterial communities with healthy ones, often used to treat recurrent C. difficile infections in humans [17].

This method was used in dogs with CE of IBD type, and showed clinical improvement [18], [19] that can help with healing of the intestine [20].

The prognosis of the sick individual can vary as well, depending on the severity of the disease and the responding of the individual's intestine [20].

2.1.3. CE in dogs and its multifactorial causes

The pathophysiology of CE in canines is multifactorial and remains incompletely understood, mirroring the complexities seen in human IBD. The immune system plays a significant role, where an inappropriate immune response to intestinal antigens, including dietary components and the gut microbiota, leads to chronic inflammation [21].

Genetic factors are also implicated, with breeds such as: *Boxers, Norwegian Lundehunds, English Bulldogs, Irish Setters, Rottweilers, Shar Peis, German Shepherds, Basenjis.*

Those genetic breeds are showing a higher predisposition to developing CE [21]

Dysbiosis, or an imbalance in the gut microbial population, has been observed in dogs with CE. Moreover, environmental factors such as diet, stress, and exposure to pathogens are thought to trigger or exacerbate the condition in canines as well [21].

In canines, it is divided into four groups[22]

(with sub-groups within it) on the base of response to treatment, as seen in figure 2:

1. FRE: food-responsive enteropathy

1.1. FR-PLE: food-responsive protein-losing enteropathy,

2. MrMRE: microbiota-related modulation-responsive enteropathy,

3. IRE: immunosuppressant-responsive enteropathy,

3.1. IR-PLE: immunosuppressant-responsive protein-losing enteropathy,

4. NRE: non-responsive enteropathy

4.1. NR-PLE: non-responsive protein-losing enteropathy.

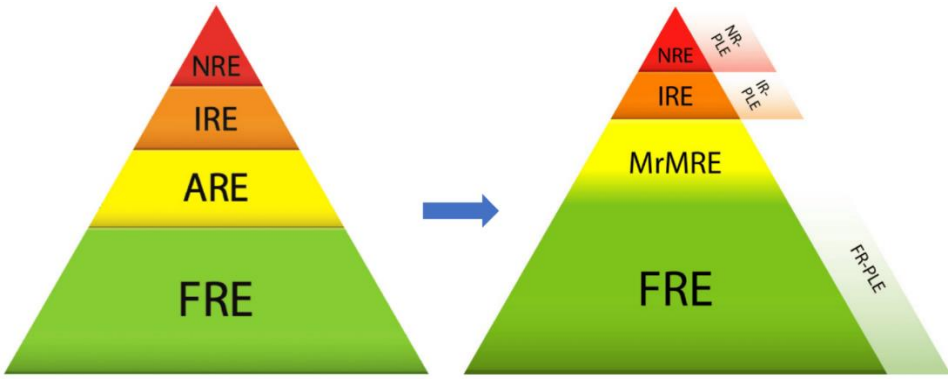

Figure 2

Classification of CE in canines

[Dupouy-Manescau et al., 2024 [22]]

In dogs, the identification and guidelines are similar to human IBD protocols

The CE may result in clinical presentations such as: chronic vomiting, hemorrhagic or non-hemorrhagic diarrhea and weight loss.

In contrary to humans, it is important first to rule out food allergy and to change the food, in order to rule out CE in dogs [23].

In case of continuous disease, it is important to check and rule out IBD in dogs.

it is important to diagnose the intestine histopathologically by an endoscope.

Biopsy results analyzed and can confirm IBD by the type of inflammation in the lamina propria of the small and/or large intestine.

Similar to humans, the cause of CE can be multifactorial: from nutritional insufficiency [24], [25], stress and anxiety [26], lifestyle management [27] and genetic factors [28]

The treatment for CE disease can vary, depending on the level of the CE [29]:

In case of FRE, the treatment will be a nutritional change and ruling out of allergens [29]

In case of MrMRE (previously "ARE"), the treatment will include using of probiotics [29]:

In case of IRE, immunosuppressant and corticosteroids will be used [29]

The treatment for IBD disease in canines varies from: corticosteroids to immunosuppressants medicines, depending on the severity of the disease and previous treatments. Nutritional

treatment and administration of a special diet is also required in order to help with the healing of the intestine and microbiome modification [29]

The prognosis of the sick animal can vary as well, depending on the severity of the disease and the responding of the animal's intestine [30]
In the following paragraphs, some characteristics of canine CE will be presented. We focus on relationships with pathogens as well as a canine specific CE form, called: Protein-losing enteropathy (PLE). It is described also through the presentation of Yorkshire terrier enteropathy.

Giardia and parvo infections in canines, may risk and lead to development of CE disease, by causing a severe damage of the intestinal barriers [31, 32]

2.1.4. Giardia infections in canines and their susceptibility to cause CE

Some enteric parasites, such as *Giardia*, significantly alter the gut microbiota of dogs [33] *Giardia intestinalis*, is an intestinal parasite, a protozoan agent. It is associated with diarrhea, causing some of the most notable changes. In naturally infected 9-week-old puppies, those with a high fecal *Giardia* cyst load exhibited greater bacterial richness compared to puppies with a low cyst load [34]

Furthermore, *Giardia* cyst shedding was positively correlated with an increase in bacterial groups linked to human gut diseases, such as *Anaerobiospirillum succiniproducens*, which weaken the intestinal mucus barrier. This weakening facilitates *Giardia's* ability to penetrate the barrier and promotes the colonization of other enteric pathogens [34].
In 22-week-old puppies, a high *Giardia* cyst load was also associated with a decline in *Lactobacillus johnsonii*, a bacterium specific to young dogs. This bacterium is thought to play a vital role in early gut health by modulating the immune system, inhibiting pathogens, and adhering to epithelial cells [35] [36]

2.1.5. Canine parvovirus as a suspected agent to cause CE

Canine parvovirus (CPV2) is a virus from *Parvoviridae* family**,** attacking Canidae.
in dogs it is causing weaning diarrhea, hemorrhagic enteritis, and death in puppies [37]

It was shown by a study of naturally infected 6-week-old puppies, that *CPV2* led to severe gut microbiota changes, including an increase in *Proteobacteria* and a decrease in *Bacteroidetes* and *Fusobacteria*, which are essential to microbiome health [38]

Similar bacterial shifts have been observed in adult dogs with inflammatory bowel disease and puppies infected with *Giardia*, indicating that these changes reflect dysbiosis [38]

2.1.6. CE in Yorkshire terriers

Chronic enteropathy in Yorkshire terriers is slightly different from other breeds of dogs.
In CE of Yorkshires, we will mostly see this CE as “protein losing enteropathy (PLE)”.
The exhibition of symptoms includes: Chronic diarrhea, vomiting, weight loss, rarely muscle tremors or seizures, as well as ascites, pleural effusion, peripheral edema and severe panhypoproteinemia (low albumin and globulin levels) by blood test [39]

In some cases, Hypovitaminosis D can be seen as well in blood tests [39].

A distinct sonographic finding in some cases is mucosal speckling, linked to lacteal dilatation, as seen in figure 3.
Lymphangiectasia, accompanied by increased mucosal cellularity, is a frequent histopathological finding in dogs with PLE, both in Yorkshire Terriers and other breeds.
Additionally, intestinal crypt abnormalities, described as crypt abscesses, dilated crypts, or cystic crypts, have also been noted in Yorkshire Terriers with PLE [40].

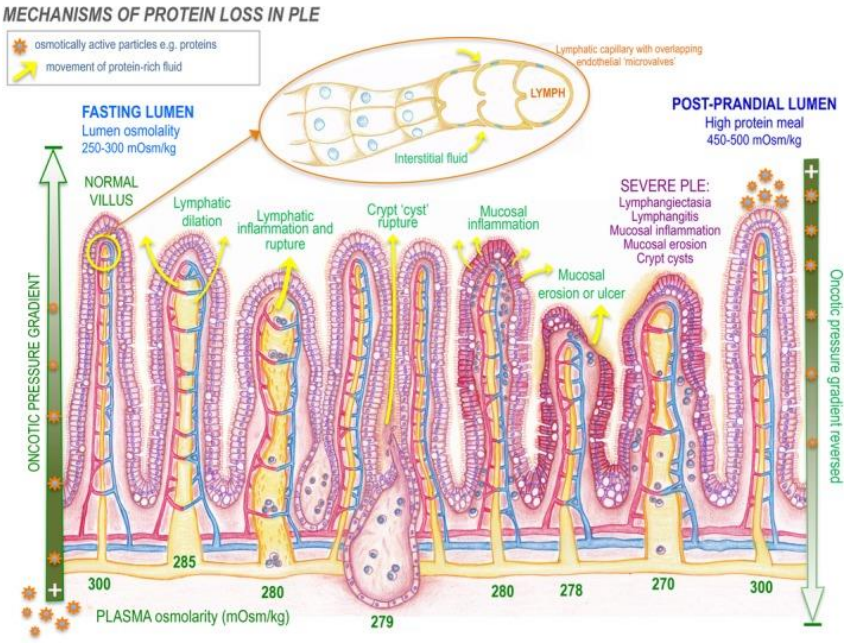

Figure 3
Diagrammatic representation of "PLE"
[Craven et al., 2019[40]]

2.2. Changing in composition of the canine microbiome by age

The intestinal microbiota refers to all the living microorganisms in a specific environment, like bacteria, archaea, fungi, protists and algae. [41] [142]

The GI microbiome includes the microbiota, their functions, and the genetic elements of nonliving organisms. Viruses, phages, and extracellular DNA being non-living, are not considered part of the microbiota, but are included in the microbiome. Molecules produced by those organisms and by the host are also part of the microbiome, including the nucleic acids, proteins, lipids, polysaccharides and metabolites [41]

The intestinal microbiome is considered a highly complex microbial ecosystem, consisting of several hundred different bacterial genera and more than a thousand bacterial phylotypes, as seen in figure 4.

The intestinal microbiome consists of approximately 10 times more microbial cells than the number of host cells, and the microbial gene pool is 100-fold larger compared to the host gene pool [41]

The ecosystem of the intestinal microbiome plays a crucial role in regulation of host health and immunity, as demonstrated in many studies in humans, animal models such as dogs and others [41]

The microbiome develops during pregnancy and early puppyhood [42], and differs by the years of the puppy, to adulthood.

In puppies and adults, the gut microbiome is composed of the followings [43]:

2.2.1. Core phyla of fecal bacterial community in heathy dogs

Firmicutes: *Clostridium spp, Lactobacillus spp, Enterococcus spp, Ruminococcus spp, Faecalibacterium spp.*

Bacteroidetes: *Bacteroides spp, Prevotella spp, Parabacteroides spp.*

Proteobacteria: *Escherichia spp (E coli)****,*** *Salmonella spp, Campylobacter spp.*

Actinobacteria: *Bifidobacterium spp, Corynebacterium spp.*

Fusobacteria: *Cetobacterium spp, Fusobacterium spp, Hypnocyclicus spp, Ilyobacter spp, Propionigenium spp.*

Other microorganisms:

Fungi: *Candida spp, Saccharomyces spp.*

Viruses: *Bacteriophages, canine enteric viruses.*

Archaea: *Methanobrevibacter spp.*

However, there are differences in the amount/percentages of the bacterial composition by the age of the canine [41]

And even the microbiome can vary by the type of birth (vaginal vs cesarean) [41]

In puppies, the microbiome composition of ***Proteobacteria*** is higher in about 10%-30% due to early microbial colonization and immune development [41]

Fusobacteria however was minimal in puppies and increased in adults, reflecting dietary shifts [41]

Bacteroidetes: increased when dogs weaned and start eating solid foods. *Bacteroides* are aiding in carbohydrate digestion and therefor this shift occurred [41]

2.2.2. Factors Influencing Changes from puppyhood to adulthood

The transition from milk to solid food influences microbial diversity, *lactobacillus* bacteria will decrease, due to changing from high-lactose diet, consumed by breastfed dogs [44]

2.2.3. Dysbiosis of the gut microbiome

The microbiome of canines will change after weaning. The change is dependent on their diet, their environment and habitat, stress factors and occurrence or illnesses with some infectious micro-organisms such as bacteria, parasites and viruses that can shift and change the microbiome completely [45], [41]

This change may lead to “dysbiosis”.

Dysbiosis is defined as imbalance or interference to the composition of the gut microbiome and its microorganisms [46]

Dysbiosis in some cases can be referred to as “bacterial overgrowth dysbiosis”, where specific bacterial taxa proliferate excessively, often disrupting the ecological balance and resulting in competitive exclusion of other commensal microbes, metabolic dysregulation, and potential pathogenicity. [46]

Dysbiosis can lead to a disfunction of the intestines, malnutrition, malabsorption and IBD in humans [47] or CE in dogs [48]

In order to identify and diagnose dysbiosis in dogs, a diagnostic tool named: “Dysbiosis index” (DI) was created, and its role is to identify shifts in the microorganisms value, according to healthy individuals, gathered by many years of research [49]
It is a performed by quantitative PCR-based assay that is used to evaluate canine microbiome compositions via fecal samples of GI patients. This method uses the accumulated numbers of 7 “core” fecal bacterial taxa as well as the total bacterial abundance. These core bacteria taxa are mostly altered in GI disorders such as chronic enteropathies (CE) and by other microbiome alterations such as drug-use, specifically antibiotics. The changes and alterations in numbers of the microbiome by the index of the DI, can predict a dysbiosis or IBD [50].

DI has a -10 to 10 scale in dogs. A negative number means a healthy microbiome, while a positive number from 2 and above it, is considered “dysbiosis”.

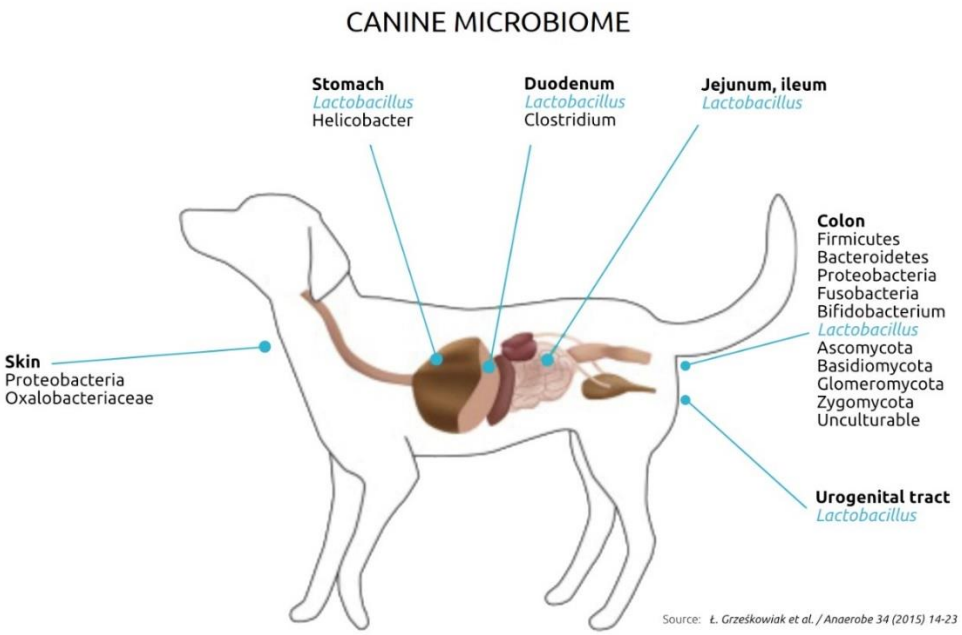

Figure 4
Composition of the canine microbiota
[L. grzeskowiak et al.,2015 [142]]

2.3. Metagenomics and next generation sequencing (NGS) as diagnostic methods of the microbiome

Metagenomics is a field in science that analyzes DNA of microorganisms in their natural environment. It is performed by sampling of the desired part, to DNA/RNA extraction.

The extracted DNA/RNA is fragmented to create a "library/profile" of the genome. After this process the fragmented material will be ready for sequencing [51]

The used technology nowadays, is "Next generation sequencing (NGS) "

2.3.1. Next generation sequencing (NGS)

A modern, high-technology method, used for rapid analysis of million sequences of DNA/RNA. It is helping with massive sequencing of several microorganisms or for complexed environment such as the microbiome [52]

There are 3 main methods in NGS [53]:

1. Shotgun- this is a method that targets all the DNA and gives species level identification of an entire environment. The Shotgun method can help with identification of unknown species.

2. Amplicon- this is a method that targets a specific genetic region of the microorganism, such as the ribosomal genetic area of the bacteria, called: "16S rRNA". This method gives specific data about microorganisms and is considered faster and easier to analyze.

3. Illumina- this is a method that combines the shotgun and amplicon methods, by creating short-reads of the required sequence.

2.3.2. The importance of NGS in microbiome studies [54]

NGS is important for microbiome studies due to its ability to identify many different microorganism strains shortly, from a small sample.

NGS can help with revolutionizing microbiome research by providing high-resolution data on microbial diversity, composition, and functional potential, which is critical for understanding microbiome-associated health and disease dynamics.

Other methods such as bacterial cultures are irrelevant to use, due to the anaerobic conditions that the microorganisms of the intestines are requiring, which cannot be performed in labs or even to be cultivated.

2.3.3. 16S Ribosomal RNA function and its role in Bacterial Mapping [55]

The 16S ribosomal RNA is a part of the 30S small subunit of prokaryotic ribosomes. Ribosomes are organelles responsible for protein synthesis in cells.

The 16S rRNA plays a crucial structural role in the ribosome, helping to maintain its shape and stabilize the interactions between other ribosomal components.
The 16S rRNA is directly involved in the binding of messenger RNA to the ribosome. It helps to correctly position the mRNA so that the ribosome can read its sequence during translation.
The 16S rRNA contains a sequence called the “Shine-Dalgarno” sequence in prokaryotes, which is complementary to a sequence in the mRNA. This interaction helps to initiate protein synthesis.
The 16S rRNA gene contains dense and conserved regions of sequences with different regions within it. The conserved regions are similar across different bacterial species, while the variable regions differ significantly. The variable regions are crucial to bacterial mapping and bacterial identification.
The 16S rRNA gene is present in almost all the bacteria.
By analyzing the 16S rRNA gene sequences, it is possible to construct phylogenetic trees that show the evolutionary relationships between bacterial species, which helps in better understanding of the microbiome and its environment.

Aims

The goal of this thesis is to deepen our understanding of chronic enteropathies (CE) in dogs- a condition that profoundly affects not only the health and well-being of our canine companions but also their bond with us.

By analyzing the microbiome of healthy and CE-affected dogs, this work aims to uncover the specific bacterial imbalances contributing to the disease. We hypotheses that we can identify microbial patterns that can serve as markers for early detection and guide tailored treatments.

By focusing on breeds like Yorkshire Terriers and comparing them with mixed breeds, this research aims to uncover whether certain dogs are inherently more vulnerable to CE and why. This insight could lead to more precise, breed-specific care and preventative measures.

This thesis also seeks to demonstrate how cutting-edge technologies, like next-generation sequencing, can provide faster and more accurate diagnoses.

This thesis is inspired by a deep respect for the animals who share our lives, as they face challenges imposed by our modern world, such as: stress, dietary changes, and environmental pressures.
Through this research, we hope to shed some light to a future of better diagnosing and treating of sick CE individuals.

3. Material and Methods

The 16S rRNA targeted short read sequenced datasets were obtained from the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) repository (https://www.ncbi.nlm.nih.gov/sra). The BioProject PRJNA861113 and PRJNA905458 were sequenced by Illumina, while the BioProject PRJNA235214 and PRJNA240561 by 454-pyrosequencing technology.

Bioinformatic analysis

On raw FASTQ-files quality-based filtering and trimming was performed by Trimmomatic[56] using 20 as a quality threshold. Only reads longer than 50 bp were retained. For deduplication and chimera filtering VSEARCH was used. The remaining reads were taxonomically classified using Kraken2 (k = 35)[57] with the Greengenes database (https://greengenes.secondgenome.com/). The taxon classification data was managed in R[58] using functions of package phyloseq[59] and microbiome[60]. Core bacteria was defined as the relative abundance of agglomerated counts at class level above 1% at least one of the samples. Variance based filtering followed abundance differences in bacteriome between groups were analysed by a negative binomial generalised model of DESeq2 R package [61]. According to the multiple comparisons, the FDR-adjusted p-value less than 0.05 was considered significant.

Data

The data was gathered with the use of several NCBI data base, project numbers:

1. PRJNA905458- a study [62] from university of Madrid, on 46 dogs from mixed breeds, age and sex. 2 groups were formed: Healthy control and CE diseased.
This study used duodenal samples and fecal samples. For adequate comparability we chose to use only the fecal samples.

2. PRJNA861113- a study [63] from university of Vienna, on 39 Yorkshire terries breed only, from mixed ages and sex. 3 groups were formed: Healthy control, CE diseased and CE post treatment that achieved remission. In this project we used the Healthy control and CE diseases pre-treatment only.

I used data from sequenced fecal samples gathered from both projects.
In both projects, the samples targeted the 16S ribosomal RNA gene, and the technology sequencing it that was used was: ‘Illumina amplicon”.

The reason for choosing these 2 projects was because they met our inclusion criteria: these projects were detailed enough, had control group and CE group individually, which helped us comparing within the groups.

We used one other project, but just to compare their findings in the discussion, due to its lack of information and data. We used the “pre-treatment” groups only for a model for comparison. The project is named: “PRJNA235214” [64], from Texas A&M university.

We were examining projects: “PRJNA401447” [65], “PRJNA240561” [66] as well but excluded because of them for not fitting into our thesis.

4. Results

For our thesis, we used data sets from 2 different projects:

1. PRJNA905458- a study [62] from university of Madrid, on 46 dogs from mixed breeds, age and sex, full comparison is listed in Table 1. This study used duodenal samples and fecal samples. We chose to use only the fecal samples.
In this study, 2 group were formed:

(a. Healthy group (we called it MBH- Mixed breed healthy): this group contained **12** healthy dogs from different breeds, ages and sex.

(b. IBD dogs (we called it MBCE- mixed breed chronic enteropathy): this group contained **34** sick dogs from different breeds, ages and sex.

Table 1. *Comparison of signalment, epidemiological data, and clinical scores of the dogs enrolled in study "PRJNA905458".*

Variables	**HC (*n* = 12)**	**IBD (*n* = 34)**	***p*-Value**
Age (years; mean ± SD)	5.31 ± 3.09	6.05 ± 3.47	0.519
Sex (male/female)	7/5	15/19	0.487
Fertile status (spayed or neutered/entire)	8/4	21/13	1.000
Breed (pure/mixed)	7/5	24/10	0.436
Weight (kg); median [range])	13.85 [4.50–32.80]	11.80 [2.30–44]	0.763
BCS (1–9); median [range])	5.50 [5–7]	4.00 [2–7]	0.001 *
Living with other pets (yes/no)	7/5	10/24	0.093
Habitat (indoor/50–50/outdoor)	8/0/4	25/7/2	0.025 *
CIBDAI (median [range])	0 [0]	6.5 [3–10]	<0.0001 *
CCECAI (median [range])	0 [0]	7 [3–12]	<0.0001 *
Duodenal biopsies/fecal samples	7/12	30/34	na

2. PRJNA861113- a study [63] from university of Vienna, on 39 Yorkshire terries, from mixed ages and sex full comparison is listed in Table 2
In this study, 2 group were formed:

(a. a healthy control group (we called it YTH- Yorkshire terrier healthy): this group contained **26** healthy Yorkshires from different ages and sex.

(b. Yorkshire terrier enteropathy (we called it YTCH- Yorkshire terrier chronic enteropathy): this group contained **13** sick Yorkshires from different ages and sex.

Table 2: Comparison of signalment, epidemiological data, and clinical scores of the dogs enrolled in study "PRJNA861113".

Variables	**YTE (*n* = 13)**	**Control (*n* = 26)**	***p*-Value**
Age (years; mean ± SD)	6.4 ± 1.7	6.05 ± 3.47	-
Sex (male/female)	4/9	12/14	-
Fertile status (spayed or neutered/entire)	2/4	6/8	-
Weight (kg); median [range])	3.7 kg ± 1.5 kg	3.9 ± 1.35	-
BCS (1–9); median [range])	4.3 out of 9 ± 1	5.5 ± 0.8	-
Muscle condition score (A-D)	A in nine dogs, B in one, C in two, and D in one dog	A (N = 20) or B (N = 6)	-

Variables	YTE (*n* = 13)	Control (*n* = 26)	*p*-Value
(WSAVA) scores for mild or moderate histological changes in the duodenum	median 3.5, range 2–11	NA	-
Diet	NA	Commercial diet (n=25), BARF diet (n=1)	-
CCECAI (median)	mean 9.2 ± 3	0.5 (range 0–3)	-

In YTH- Yorkshire terrier healthy, the most prominent and abundant bacteria were:

1. *Prevotella copri, Genus: Prevotella* - (log2 fold change(fc): -5.53, adjusted p-value (q=0.00)
2. *Eubacterium biforme, Genus: Eubacterium* - (fc: -3.19, q=0.00)
3. *Megamonas hypermegale, Genus: Megamonas* - (fc: -2.93, q=0.00)
4. *Bacteroides coprophilus, Genus: Bacteroides* - (fc: -2.30, q=0.01)

This data can be seen is listed as well in Table 4, Figure 6

In YTCE- Yorkshire terrier chronic enteropathy pre-treatment, the most prominent and abundant bacteria were:

1. *Clostridium perfringens, Genus: Clostridium* - (log2 fold change(fc): 2.92, q=0.00)
2. *Bacteroides fragilis, Genus: Bacteroides* - (fc: 2.92, q=0.00)
3. *Streptococcus luteciae, Genus: Streptococcus* - (fc: 2.84, q=0.01)
4. *Streptococcus alactolyticus, Genus: Streptococcus* - (fc: 2.08, q=0.01)
5. *Collinsella stercoris, Genus: Collinsella* - (fc: 0.52, q=0.65)
6. *Blautia producta, Genus: Blautia* - (fc: 0.55, q=0.63)

The decreased bacteria in this group were:

Prevotella copri, Genus: Prevotella - (fc: -5.53, q=0.00)

Eubacterium biforme, Genus: Eubacterium - (fc: -3.19, q=0.00)

Bacteroides coprophilus, Genus: Bacteroides - (fc: -2.30, q=0.01)

This data can be seen is listed as well in Table 4, Figure 6

In MBH- Mixed breed healthy, the most prominent and abundant bacteria were:

1. *Faecalibacterium prausnitzii, Genus: Faecalibacterium* - (fc: -1.33, q=0.21)
2. *Lactobacillus ruminis, Genus: Lactobacillus* - (fc: -5.63, q=0.01)
3. *Lactobacillus reuteri, Genus: Lactobacillus* - (fc: -2.43, q=0.25)

   This data can be seen is listed as well in Table 3, Figure 5

In MBCE- Mixed breed Chronic enteropathy, the most prominent and abundant bacteria were:

1. *Plesiomonas shigelloides, Genus: Plesiomonas* - (fc: 4.51, q=0.02)
2. *Salmonella enterica, Genus: Salmonella* - (fc: 4.30, q=0.02)
3. *Serratia marcescens, Genus: Serratia* - (fc: 3.79, q= 0.02)
4. *Blautia producta, Genus: Blautia* - (fc: -0.35, q=0.56)
5. *Ruminococcus gnavus, Genus: Ruminococcus* - (fc: 0.18, q=0.77)

The decreased bacteria in this group were:

Lactobacillus ruminis, Genus: Lactobacillus - (fc: -5.63, q=0.01)

Lactobacillus reuteri, Genus: Lactobacillus - (fc: -2.43, q=0.25)

Bacteroides genus – (fc: -0.87 q=0.67)

This data can be seen is listed as well in Table 3, Figure 5

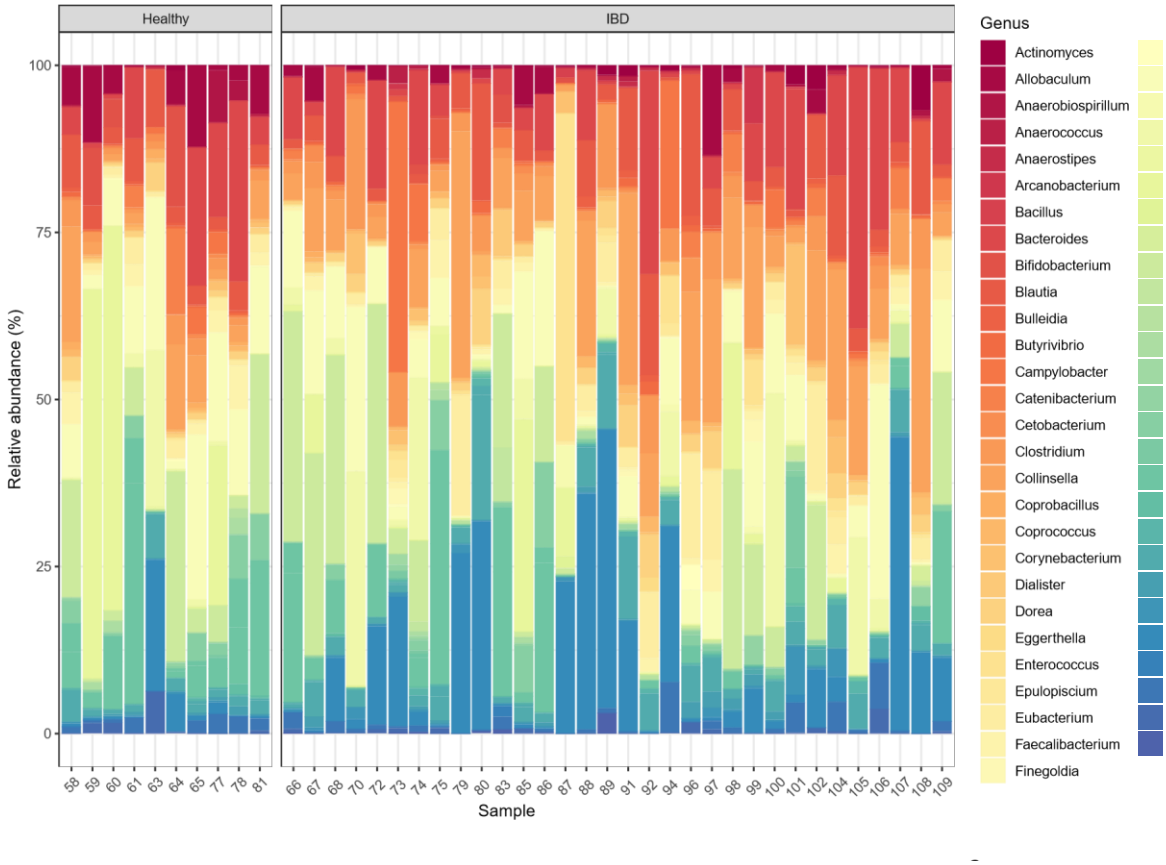

Figure 5:
Relative abundances of genera in core bacteriom from the BioProject PRJNA905458 [62].

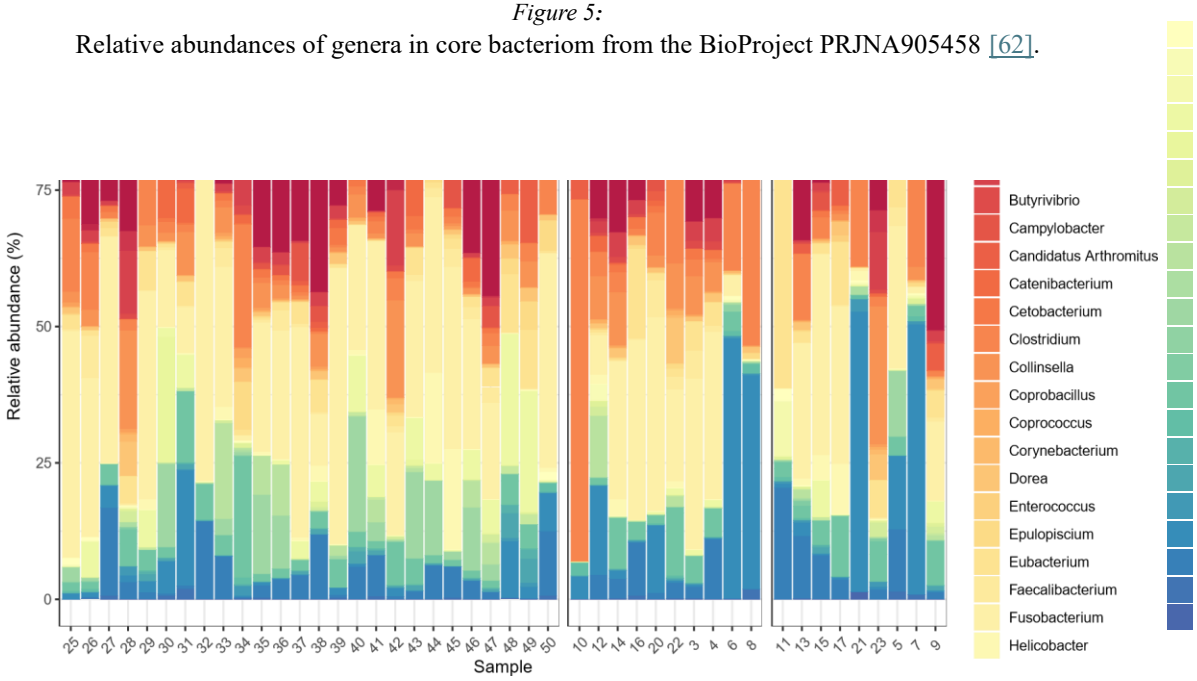

Figure 6:

Relative abundances of genera in core bacteriom from the BioProject PRJNA861113 [63]

Table 3: Fold change IBD/Healthy in PRJNA905458.

	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
Lactobacillus ruminis	6.67	-5.63	1.44	-3.91	0.00	0.01
Lactobacillus helveticus	1.91	-4.61	2.26	-2.04	0.04	0.21
Lactobacillus delbrueckii	4.82	-4.58	1.79	-2.56	0.01	0.15
Plesiomonas shigelloides	3.49	4.51	1.35	3.33	0.00	0.02
Lactobacillus manihotivorans	12.70	-4.51	2.58	-1.75	0.08	0.25
Lactobacillus salivarius	1.86	-4.45	1.81	-2.46	0.01	0.15
Corynebacterium kroppenstedtii	5.28	4.43	1.85	2.39	0.02	0.15
Salmonella enterica	5.43	4.30	1.27	3.39	0.00	0.02
Lactobacillus pontis	5.16	-4.03	2.06	-1.95	0.05	0.21
Bifidobacterium pseudolongum	5.00	-4.02	2.08	-1.93	0.05	0.21
Lysinibacillus boronitolerans	4.56	-3.91	1.26	-3.11	0.00	0.04
Variovorax paradoxus	3.41	3.82	1.47	2.59	0.01	0.15
Serratia marcescens	20.05	3.79	1.08	3.50	0.00	0.02
Lactobacillus zeae	16.76	-3.61	1.53	-2.36	0.02	0.15
Lactobacillus mucosae	16.15	-3.41	1.50	-2.28	0.02	0.15
Lactobacillus coleohominis	0.44	-3.38	3.43	-0.99	0.32	0.54
Erwinia dispersa	1.54	3.34	1.52	2.20	0.03	0.16
Klebsiella oxytoca	1.54	3.34	1.37	2.43	0.02	0.15
Lactobacillus paralimentarius	1.95	-3.33	1.97	-1.69	0.09	0.28

Trabulsiella farmeri	1.41	3.23	1.60	2.01	0.04	0.21
Bifidobacterium longum	14.25	-3.12	1.88	-1.65	0.10	0.28
Bifidobacterium adolescentis	21.66	-3.03	1.99	-1.52	0.13	0.31
Actinomyces hyovaginalis	8.23	2.85	2.84	1.00	0.32	0.54
Lactobacillus vaginalis	13.69	-2.70	1.91	-1.42	0.16	0.35
Streptococcus minor	0.98	2.68	2.42	1.11	0.27	0.47
Bifidobacterium thermacidophilum	2.10	-2.58	2.08	-1.24	0.21	0.41
Bifidobacterium breve	3.30	-2.56	1.90	-1.34	0.18	0.38
Eggerthella lenta	2.02	2.52	2.26	1.11	0.27	0.47
Lactobacillus acidipiscis	0.61	-2.50	1.58	-1.58	0.12	0.30
Sporomusa polytropa	1.08	2.49	2.02	1.23	0.22	0.41
Lactobacillus reuteri	316.89	-2.43	1.37	-1.77	0.08	0.25
Lactobacillus agilis	3.12	-2.18	1.75	-1.25	0.21	0.41
Clostridium butyricum	3.06	2.17	1.13	1.92	0.06	0.21
Aliivibrio fischeri	1.65	2.06	1.03	1.99	0.05	0.21
Corynebacterium durum	11.01	1.97	1.36	1.45	0.15	0.33
Peptostreptococcus anaerobius	43.90	1.86	0.75	2.48	0.01	0.15
Parabacteroides distasonis	0.68	1.80	1.40	1.29	0.20	0.41
Lactobacillus iners	22.72	-1.80	1.63	-1.10	0.27	0.47
Prevotella copri	1633.09	-1.73	1.08	-1.60	0.11	0.29
Alloiococcus otitis	3.61	-1.67	1.04	-1.61	0.11	0.29
Veillonella dispar	23.12	1.64	0.72	2.28	0.02	0.15

Streptococcus luteciae	408.01	1.57	1.07	1.48	0.14	0.33
Pediococcus acidilactici	1.03	1.52	1.63	0.93	0.35	0.56
Streptococcus infantis	5.06	1.51	1.20	1.26	0.21	0.41
Bifidobacterium animalis	0.83	-1.50	2.14	-0.70	0.48	0.68
Staphylococcus sciuri	13.22	1.48	0.83	1.79	0.07	0.25
Streptococcus alactolyticus	235.18	1.47	0.89	1.66	0.10	0.28
Staphylococcus epidermidis	35.79	1.42	0.62	2.31	0.02	0.15
Coprococcus eutactus	1.89	1.38	1.02	1.35	0.18	0.38
Eubacterium dolichum	159.75	1.37	0.75	1.81	0.07	0.25
Porphyromonas endodontalis	1.50	-1.37	1.52	-0.90	0.37	0.58
Faecalibacterium prausnitzii	554.98	-1.33	0.64	-2.08	0.04	0.21
Ruminococcus flavefaciens	9.84	1.11	0.57	1.94	0.05	0.21
Clostridium perfringens	291.06	1.09	0.61	1.77	0.08	0.25
Campylobacter rectus	7.68	1.05	1.66	0.64	0.53	0.71
Clostridium neonatale	2.34	1.05	1.41	0.74	0.46	0.67
Haemophilus influenzae	1.90	1.02	0.68	1.51	0.13	0.31
Collinsella aerofaciens	423.50	0.98	0.49	2.02	0.04	0.21
Anoxybacillus kestanbolensis	16.45	-0.94	0.76	-1.24	0.21	0.41
Roseburia faecis	27.42	0.93	0.58	1.62	0.11	0.29

Streptococcus anginosus	5.38	0.91	0.93	0.98	0.33	0.54
Collinsella stercoris	1762.37	0.89	0.57	1.56	0.12	0.30
Prevotella melaninogenica	13.32	-0.89	1.18	-0.75	0.45	0.67
Bacteroides plebeius	159.45	-0.87	1.02	-0.85	0.39	0.60
Lactobacillus brevis	2.98	-0.85	1.76	-0.49	0.63	0.77
Prevotella stercorea	16.95	-0.82	1.21	-0.68	0.49	0.69
Staphylococcus aureus	11.55	0.81	0.67	1.21	0.22	0.41
Neisseria cinerea	0.22	0.79	3.46	0.23	0.82	0.89
Prevotella nigrescens	15.14	-0.74	1.19	-0.62	0.54	0.72
Bacteroides eggerthii	4.01	-0.73	1.32	-0.55	0.58	0.75
Helicobacter pylori	67.55	0.71	1.60	0.45	0.66	0.77
Helicobacter hepaticus	59.53	0.70	1.54	0.45	0.65	0.77
Selenomonas noxia	4.42	-0.68	0.56	-1.22	0.22	0.41
Blautia obeum	114.47	-0.65	0.29	-2.25	0.02	0.15
Ruminococcus torques	46.63	-0.62	0.34	-1.85	0.06	0.24
Bacteroides fragilis	79.76	0.60	0.85	0.70	0.48	0.68
Cetobacterium somerae	115.65	-0.60	0.85	-0.71	0.48	0.68
Morganella morganii	0.42	0.59	0.99	0.60	0.55	0.72
Bacteroides coprophilus	29.31	-0.47	0.99	-0.48	0.63	0.77
Bacteroides barnesiae	0.97	-0.46	1.04	-0.44	0.66	0.77
Haemophilus parainfluenzae	9.36	0.42	0.65	0.64	0.52	0.71
Clostridium hiranonis	356.03	-0.41	0.53	-0.77	0.44	0.67
Megamonas hypermegale	41.09	0.40	0.95	0.42	0.68	0.78
Bacteroides ovatus	53.18	-0.40	0.81	-0.49	0.62	0.77
Ruminococcus bromii	3.48	0.37	0.73	0.50	0.62	0.77

Blautia producta	306.48	-0.35	0.37	-0.93	0.35	0.56
Prevotella tannerae	4.94	0.32	1.06	0.30	0.76	0.85
Veillonella parvula	3.51	-0.26	0.64	-0.41	0.68	0.78
Bulleidia p-1630-c5	6.33	-0.20	0.55	-0.36	0.72	0.80
Desulfosporosinus meridiei	12.63	0.19	0.51	0.38	0.70	0.80
Ruminococcus gnavus	608.27	0.18	0.40	0.45	0.65	0.77
Sharpea p-3329-23G2	0.44	-0.14	1.06	-0.13	0.90	0.95
Propionibacterium acnes	24.27	-0.11	0.43	-0.25	0.81	0.89
Leptospirillum ferrodiazotrophum	0.90	-0.07	1.38	-0.05	0.96	1.00
Dorea formicigenerans	45.07	-0.06	0.40	-0.14	0.89	0.95
Bacteroides uniformis	15.67	0.03	1.02	0.03	0.97	1.00
Bacteroides caccae	22.62	0.02	0.87	0.03	0.98	1.00
Bacteroides acidifaciens	3.96	0.02	0.99	0.02	0.98	1.00
Eubacterium biforme	679.26	-0.00	0.72	-0.01	1.00	1.00
Akkermansia muciniphila	0.05	-0.00	3.47	-0.00	1.00	1.00
Mucispirillum schaedleri	0.00	0.00	0.00	0.00	1.00	

Table 4: Fold change Diseased/Control in PRJNA861113

	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
Prevotella copri	35.64	-5.53	0.95	-5.82	0.00	0.00
Eubacterium biforme	9.22	-3.19	0.86	-3.69	0.00	0.00
Megamonas hypermegale	9.84	-2.93	0.70	-4.20	0.00	0.00
Clostridium perfringens	167.41	2.92	0.80	3.67	0.00	0.00
Bacteroides fragilis	38.50	2.92	0.78	3.73	0.00	0.00
Streptococcus luteciae	95.15	2.84	0.94	3.01	0.00	0.01
Bacteroides coprophilus	20.25	-2.30	0.76	-3.04	0.00	0.01
Streptococcus alactolyticus	12.44	2.08	0.70	2.96	0.00	0.01
Helicobacter pylori	4.28	-1.39	0.67	-2.07	0.04	0.13
Bifidobacterium longum	2.25	-1.20	0.64	-1.87	0.06	0.17
Helicobacter hepaticus	4.83	-0.96	0.67	-1.43	0.15	0.39
Ruminococcus torques	6.45	0.80	0.40	2.00	0.05	0.14
Eubacterium dolichum	143.59	0.76	0.69	1.09	0.28	0.61
Bacteroides caccae	11.48	0.64	0.59	1.09	0.28	0.61
Blautia producta	39.66	0.55	0.60	0.91	0.36	0.63
Bacteroides plebeius	74.37	-0.54	0.86	-0.63	0.53	0.67
Collinsella aerofaciens	2.92	-0.54	0.55	-0.99	0.32	0.63
Clostridium butyricum	3.71	-0.52	0.65	-0.80	0.42	0.65
Collinsella stercoris	108.29	0.52	0.74	0.70	0.48	0.65
Dorea formicigenerans	6.29	0.49	0.51	0.96	0.33	0.63
Clostridium neonatale	3.86	0.49	0.62	0.78	0.43	0.65
Parabacteroides distasonis	3.59	-0.48	0.64	-0.75	0.45	0.65
Faecalibacterium prausnitzii	41.39	0.47	0.76	0.62	0.54	0.67
Cetobacterium somerae	40.08	-0.47	0.64	-0.73	0.46	0.65
Clostridium hiranonis	29.59	0.46	0.49	0.92	0.36	0.63

Plesiomonas shigelloides	4.72	0.36	0.63	0.57	0.57	0.67
Ruminococcus gnavus	117.26	0.27	0.57	0.47	0.64	0.74
Blautia obeum	7.10	0.21	0.53	0.40	0.69	0.76
Bacteroides eggerthii	4.05	-0.07	0.52	-0.13	0.90	0.96
Serratia marcescens	6.83	-0.02	0.61	-0.04	0.97	0.98
Leuconostoc mesenteroides	1.33	0.02	0.60	0.03	0.98	0.98

Table 5: Fold change AHD/NHD in PRJNA235214

	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
Prevotella copri	147.82	23.52	2.54	9.27	0.00	0.00
Streptococcus alactolyticus	35.96	8.21	3.46	2.37		
Lactobacillus ruminis	26.70	-7.45	3.93	-1.90		
Streptococcus luteciae	119.87	6.82	3.18	2.15		
Ruminococcus torques	10.40	3.28	1.59	2.06	0.04	0.32
Bacteroides plebeius	75.21	-2.85	1.53	-1.86	0.06	0.32
Bacteroides coprophilus	27.78	2.50	1.91	1.31	0.19	0.60
Blautia obeum	3.83	-2.33	1.33	-1.74	0.08	0.32
Lactobacillus acidipiscis	0.83	2.09	4.01	0.52	0.60	0.83
Dorea formicigenerans	5.79	-1.55	1.32	-1.18	0.24	0.65
Clostridium perfringens	492.43	1.40	1.46	0.96	0.34	0.80
Lactobacillus salivarius	2.80	1.37	3.97	0.35		
Cetobacterium somerae	32.06	-1.23	1.58	-0.78	0.44	0.83
Faecalibacterium prausnitzii	26.04	-1.22	1.69	-0.73	0.47	0.83
Collinsella stercoris	35.70	1.19	0.69	1.72	0.08	0.32
Lactobacillus reuteri	1.31	-0.90	4.02	-0.22	0.82	0.92
Prevotella melaninogenica	2.20	0.82	2.22	0.37	0.71	0.90
Clostridium hiranonis	163.53	0.58	1.14	0.51	0.61	0.83
Eubacterium dolichum	6.45	-0.54	1.96	-0.27	0.78	0.92
Blautia producta	38.53	0.41	0.68	0.61	0.54	0.83
Ruminococcus gnavus	40.81	-0.35	0.64	-0.55	0.58	0.83
Bacteroides caccae	4.46	-0.29	1.99	-0.15	0.88	0.93
Eubacterium biforme	27.57	-0.06	1.65	-0.04	0.97	0.97

5. Discussion

The intestinal microbiota refers to all the living microorganisms in a specific environment, like bacteria, archaea, fungi, protists and algae [41].

The microbiome plays a critical role in maintaining gut health by regulating immune responses, aiding digestion, and protecting against harmful pathogens. In IBD/CE, an imbalance in the microbiome (dysbiosis) contributes to inflammation and disease progression, with altered bacterial composition and diversity affecting the gut's immune and metabolic environment [47]

The understanding of the complex microbiome is partially understood. Monitoring and understanding the exact causes of microbiome alterations or dysbiosis in canine chronic enteropathy (CE) remains challenging. The current treatment approaches for CE are not standardized, and there is a lack of sufficient knowledge regarding the disease's pathogenesis and effective management strategies, highlighting the need for further research to develop more targeted and unified treatment options [3]

In order to control and manage this disease better, it is needed to find a better solution or mechanism that can target the disease completely, research of the microbiome can help us with understanding of the "missing parts" in this complexed "puzzle."

The study [62] of University of Madrid was on 46 dogs from mixed breeds, age and sex. The dogs were divided into 2 groups: Healthy control and CE diseased.
This study used duodenal samples and fecal samples.
Their conclusion found significant differences in fecal microbiota composition and diversity between dogs with IBD and healthy dogs, while only minor changes were observed in duodenal-associated microbiota. Fecal samples, being more accessible and reliable for identifying bacterial taxa as potential biomarkers, are recommended for future research**.**

The study [63] of University of Vienna was on 39 Yorkshire terries breeds only, from mixed ages and sex. 3 groups were formed: Healthy control, CE diseased and CE after remission.
Their conclusion revealed that dogs with YTE exhibit gut dysbiosis, marked by significant beta diversity changes but no alterations in alpha diversity, indicating shifts in bacterial species without major changes in richness. Even dogs in remission showed persistent dysbiosis, with

bacterial compositions closer to the YTE group than healthy controls, highlighting the need for further microbiome characterization to better understand and address these changes.

The reason for choosing these 2 projects was because these projects were detailed enough, had control group and CE group individually, which helped us comparing within the groups.

Another project that we chose partially to use, was project named: “PRJNA235214” [58], from Texas A&M university. We used some data from this project because it had an important microbiome information from 3 groups:
Healthy control(H), Acute hemorrhagic diarrhea (AHD), Non-hemorrhagic diarrhea (NHD). However, we could use the project data only for comparison with significant limitations as this project had missing data about the groups, about the treatment the dogs got, and about their breeds, species and final conclusions of the authors.

In YTH- Yorkshire terrier healthy, the most prominent and abundant bacteria were:

1. *Prevotella copri, Genus: Prevotella*
2. *Eubacterium biforme, Genus: Eubacterium*
3. *Megamonas hypermegale, Genus: Megamonas*
4. *Bacteroides coprophilus, Genus: Bacteroides*

In YTCE- Yorkshire terrier chronic enteropathy pre-treatment, the most prominent and abundant bacteria were:

1. *Clostridium perfringens, Genus: Clostridium*
2. *Bacteroides fragilis, Genus: Bacteroides*
3. *Streptococcus luteciae, Genus: Streptococcus*
4. *Streptococcus alactolyticus, Genus: Streptococcus*
5. *Collinsella stercoris, Genus: Collinsella*
6. *Blautia producta, Genus: Blautia*

In MBH- Mixed breed healthy, the most prominent and abundant bacteria were:

1. *Faecalibacterium prausnitzii, Genus: Faecalibacterium*
2. *Lactobacillus ruminis, Genus: Lactobacillus*
3. *Lactobacillus reuteri**,** Genus: Lactobacillus*

In MBCE- Mixed breed Chronic enteropathy, the most prominent and abundant bacteria were:

1. *Plesiomonas shigelloides, Genus: Plesiomonas*
2. *Salmonella enterica, Genus: Salmonella*
3. *Serratia marcescens, Genus: Serratia*
4. *Blautia producta, Genus: Blautia*
5. *Ruminococcus gnavus, Genus: Ruminococcus*

PRJNA235214- AHD and NHD groups

AHD group:

1. Turicibacter genus

2. Streptococcus genera

3. Sutterella genus

4. Clostridium perfringens, Genus: Clostridium

5. Fusobacterium nucleatum, Genus: Fusobacterium

NHD group:

1. Streptococcus genera

2. Clostridium perfringens, Genus: Clostridium

3. Fusobacterium nucleatum, Genus: Fusobacterium

This data can be seen is listed as well in Table 5

YTH group:

1. *Prevotella copri-* associated with normal and healthy gut flora in both humans and dogs. *Provetella copri* is a Gram-negative, non-spore-forming, anaerobic bacterium. It is commonly found in the human gastrointestinal tract, specifically in the colon [67]
Prevotella copri is involved in various metabolic processes, including the fermentation of dietary fibers and carbohydrates. It can also produce short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate, which have important roles in gut health [68].

Prevotella copri is described as an important bacterium that helps with healthy gut flora. According to the following data [67], [68], it was discovered that presence of *Prevotella copri* is linked to healthy microbiome, and it was lacking in IBD patients both human and canine.

On the other hand, in a few other research, it was described as a bacterium that can promote Colitis in mice [69], and rheumatoid arthritis in humans [70]

. So, it is still questioned for its benefaction.

2. *Eubacterium biforme-* a Gram-positive, anaerobic, non-spore-forming, rod shape bacterium commonly found in the human and canine [71] gut microbiota. It is a member of the *Firmicutes* phylum. *Eubacterium biforme* ferments carbohydrates to produce short-chain fatty acids (SCFAs).

This bacterium is associated with gut health in humans and in canines [72]
[65]

3. *Megamonas hypermegale* - an anaerobic, rod shaped, Gram-negative bacterium from the *Bacteroidetes* phylum.
Part of the human and canine microbiome [73]
It plays a role in carbohydrate fermentation and produces short-chain fatty acids (SCFAs)

This bacterium is associated with gut health in canines [74]

4. Bacteroides coprophilus - is a species of bacteria that belongs to the *Bacteroides* genus. Gram-negative, rod-shaped bacteria, obligate anaerobic bacteria commonly found in the intestines of animals [43] and humans [75]

Bacteroides coprophilus, like many other Bacteroides species, is associated with the production of short-chain fatty acids (SCFAs).

It was found that this bacterium helps in remission in human ulcerative colitis patients that were implanted with this bacterium that was implanted in Fecal microbiome transplantation method (FMT) [76]

YTCE group:

We couldn't find an overlap or correlations between the two groups of healthy dogs (YTH, MBH). We may assume that it is because of the differences of the microbiome of the Yorkshire terriers to the other breeds of dogs.
In microbiome research of Yorkshires with CE(PLE), the abundant healthy bacteria were: *Clostridium hiranonis* and *fusobacterium* genus, while it was in minimal amount in PLE group [137]

We can assume that Yorkshire terriers may have breed specific microbiota pattern that is different from the average. And we can assume also, that it may predispose them to PLE.

1. *Clostridium perfringens*- associated with IBD group gut flora in both humans, dogs and cats. Especially in IBD patients [77].

Clostridium perfringens is a Gram-positive, anaerobic bacterium belonging to the genus *Clostridium* within the family *Clostridiaceae*. It is rod-shaped and forms spores, which allow it to survive in harsh conditions. It is found in normal and healthy gut flora [78] but can cause diseases when overgrowth occurs and by toxin releasing in humans and canines [79], [80].

Pathogenicity: *Clostridium perfringens* is known for its ability to produce toxins that can cause tissue damage and disease. It is a common cause of foodborne illness, as well as other infections.

There are several types of toxins that *clostridium perfringens* is producing, classes as A-G toxins [81].

It is commonly found in the gastrointestinal tract of humans and other animals, where it is considered a commensal bacterium, meaning it typically lives in harmony with its host.

Clostridiaceae bacteria in dogs play a role in protein digestion, with their abundance correlating with dietary protein intake. Specifically, *Clostridium perfringens* within this family is involved in the butyrate synthesis pathway, producing butyrate from protein, which differs from its role in rats' large intestines. This suggests a unique metabolic function for *Clostridiaceae* in carnivores [43].

Since *clostridium* species can severely effect both humans and animals, it is well investigated in order to find a solution to distinguish between useful and harmful bacteria from those species [77], [82]

2. *Bacteroides fragilis* - is a species of bacteria that belongs to the *Bacteroides* genus. Gram-negative, rod-shaped bacteria, obligate anaerobic bacteria commonly found in the intestines of animals [83] and humans [84]
Bacteroides fragilis on the contrary to its gut health associated genus *"Bacteroides"*, *Enterotoxigenic Bacteroides fragilis (ETBF)*, a molecular subclass of the common human commensal, *B. fragilis*, has been associated with IBD in humans [85], [86]
There was minimal information about this bacterium in CE dogs,

However, this bacterium was found as a biofilm producing agent in intestines of mixed dog breeds. The biofilm that this bacterium is producing can contribute to antibiotic resistance [87]

Our finding in Yorkshires may lead us to a new causative agent of breed specific Yorkshire terrier enteropathy.

3. *Streptococcus luteciae* – Gram-positive, cocci shaped, facultatively anaerobic bacterium of the *Streptococcus* genus.

This bacterium is not part of the intestinal microbiome, but of the skin microbiome in humans. [88]

It was found in IBD human patients. [89]
However, in **healthy** canine patients, this bacterium was used as part of the bacterial composition in fecal microbiota transplantation (FMT) capsules, in order to investigate the microbial changes in the dogs [90].

Our finding about this bacterium as a possible harmful agent in Yorkshires, may help in understanding of PLE.

4. *Streptococcus alactolyticus*- Gram-positive, cocci shaped, facultatively anaerobic bacterium of the *Streptococcus* genus.

This bacterium is not part of the intestinal microbiome of humans, but of canines. [91]

It was found as the most prominent bacterium in fistulated canines [91] and in sclerosing cholangitis human patients. [92]

Fistulas are abnormal openings in the intestine, caused by several intestinal interferences such as chronic inflammation, surgery etc. [141]
The abundance of this bacterium in fistulated dogs may contribute to intestinal damage that can lead into fistulae formation. Our findings of this bacterium abundantly in YTCE group may be a key factor in further investigation of CE.

5. *Collinsella stercoris*- Gram-positive, anaerobic, rod-shaped bacterium of the *Actinobacteria* phylum. Contributes to intestinal cholesterol absorption and triglyceride synthesis. [93]

It was found that an over-growth of this bacterium, can cause intestinal dysbiosis in canines. [94]

6. *Blautia producta*- a Gram-positive, obligate anaerobe, coccobacillary shaped of the *Lachnospiraceae* family.
This bacterium is part of the human microbiome [95] and other mammals. [96]

In humans this bacterium is inconclusive, in some research it is associated with intestinal inflammation [97] and metabolic worsening in kids [98], while in other research it is associated with mucosal health. [99]

In canines, it is associated with gut dysbiosis and mitral valve disease. [100]

MBH group:

We couldn't find an overlap or correlations between the two groups of healthy dogs (YTH, MBH). We may assume that it is because of the differences of the microbiome of the Yorkshire terriers to the other breeds of dogs.

1. *Faecalibacterium prausnitzii*- Was found in abundant amount in healthy patients, both human [101] and canine [102], and was absent or in minimal amount in IBD group patients. An important bacterium that is being investigated by many gastrointestinal researchers of humans and animals around the world.

Faecalibacterium prausnitzii is a gram-positive, anaerobic, non-spore-forming bacterium. It is a member of the *Firmicutes* phylum and is commonly found in the human gastrointestinal tract, particularly in the colon.

F. prausnitzii is considered a beneficial bacterium and is one of the most abundant species of bacteria in the human gut microbiota. It plays a significant role in humans maintaining gut health and has been associated with potential anti-inflammatory effect, if used as probiotics [103].

2. *Lactobacillus ruminis*- is a gram-positive bacterium, rode shaped, obligatory anaerobe, it is producing fermenting carbohydrates into lactic acid, which helps to inhibit harmful pathogens, support immune function, and contribute to the overall balance of the intestinal microbiota [104]. This bacterium is a part of the gut microbiome in humans, canines and other mammals [105], [106].

This bacterium is used as a probiotic strain as an immunomodulator that works on TNF-alpha (a chemical messenger that induces inflammatory processes). [107]

***3. Lactobacillus reuteri*-** is a gram-positive bacterium, rode shaped, obligatory anaerobe, it is producing lactic acid, and part of the gut microbiome in humans, canines and other mammals [108]

This bacterium is used commonly in probiotics. It is a well-known strain that is responsible for gut health both in humans [108], canines [109] and other mammals such as pigs [110]

MBCE group:

1. *Plesiomonas shigelloides*- a gram-negative bacterium, rod shaped, facultative anaerobic, can be found in fresh water and in fish and amphibians [111]

This bacterium can cause chronic diarrhea in humans [112], and was found in dogs and other animals, but without any information of its effects on dogs [113]

This bacterium is being suspected nowadays as a zoonotic agent from animals to humans [114]

2. *Salmonella enterica*- a gram negative, facultative anaerobe, rod shaped bacterium, belongs to the *Enterobacteriaceae* family.

It is not part of the human or the canine microbiome [115]

Canines can be an asymptomatic carrier of *salmonella* which can lead to risk factors such as diarrheal disease in canines [116], or zoonotic infection in humans [116]

In humans, *salmonellosis* is linked with ulcerative colitis [117]

In canines, it was linked as a cause of microbiome imbalance that can lead to chronic enteropathies [118]

3. *Serratia marcescens*- a gram-negative, facultative anaerobic bacterium, rod shape, belonging to *Enterobacteriaceae* family. Serratia is not a part of the gut microbiome [119], and the species *"Marcescens"* is being investigated for its pathogenic effects [119]
In humans it is suspected in colitis and enteritis in general [120], while in canines it is suspected in complications of parvo-virus enteritis from colonization in IV catheters [121] and cutaneous infections [122]

In human research, it was described as a pathogen with high antibiotic resistance, and production of beta-lactamase enzyme, that destroys the "beta-lactam" antibiotics mechanism. [136] This gives light in understanding of the bacterium behavior, and its potential harmful effects towards the microbiome and dysbiosis.

4. *Blautia producta-* a Gram-positive, obligate anaerobe, coccobacillary shaped of the *Lachnospiraceae* family.
this bacterium is part of the human microbiome [95] and other mammals [96]

In humans this bacterium is inconclusive, in some research it is associated with intestinal inflammation [97] and metabolic worsening in kids [98], while in other research it is associated with mucosal health [99]

In canines, it is associated with gut dysbiosis and mitral valve disease [100]

5. *Ruminococcus gnavus-* a gram positive, obligatory anaerobe, cocci shaped bacterium, belongs to the phylum *Firmicutes.*
It is part of the human gut microbiome [127] and produces short chain fatty acids (SCFA). [128]

According to research nowadays, it is associated with Crohn's disease in humans by producing an inflammatory polysaccharide [127]

In canines, it was a prominent component in the fecal microbiome of dogs with "intestinal lymphangiectasia" [123] and as a part of microbiome alteration of dogs with parvo-virus [33], [129]

PRJNA235214- AHD and NHD groups

AHD group:

1. Turicibacter
2. Streptococcus genera
3. Sutterella,
4. Clostridium perfringens
5. Fusobacterium nucleatum

NHD group:

1. Streptococcus genera
2. Clostridium perfringens
3. Fusobacterium nucleatum

We saw that in those acute enteropathy conditions, some harmful bacteria are the same as in chronic CE group, such as streptococcus genera and clostridium perfringens.
The special bacteria that we wanted to emphasis are: *Turicibacter, Sutterella genus, Fusobacterium nucleatum.*

We couldn't find in the fecal samples these bacteria, and we may assume that those are linked to acute enteropathies.
However, comparing to other acute enteropathies microbiome sample, it showed that in acute enteropathies, *E. coli* was the most abundant bacterium [138].

Fusobacterium nucleatum- Was found in both AHD and NHD groups sporadically without any significance to certain age groups. In healthy patients it was in small amounts.

Fusobacterium nucleatum is a species from *Fusobacterium* family of anaerobic bacteria, gram-negative, non-spore-forming bacilli commonly found in the oral cavity of both human and canine. [130]

In recent years, there has been increasing interest in studying *Fusobacterium nucleatum* and its potential role in various diseases including colorectal cancer and inflammatory bowel disease (IBD) in humans [131].

However, in dogs there's no data about enteropathies caused by this bacterium, but in dogs it is linked to periodontal disease. [132]
This information gives us new insights about this bacterium, its habitats and the species differences, as a cancer inducer in humans in contrary to canines [105].

Turicibacter - a genus of bacteria belonging to the family *Erysipelotrichaceae* within the phylum *Firmicutes*. These bacteria are anaerobic, gram-positive, rod-shaped organisms.
It is considered as a healthy and beneficial bacterium in microbiome of both humans and dogs [44], [49], [125]
However, its role is inconclusive. In other human research [126], it was associated with Ulcerative colitis, which is a chronic condition in contrary to canine acute hemorrhagic diarrhea**.**

Therefore, this bacterium remains a mystery, it is unknown yet if it is a healthy or harmful bacterium, or if its risk potential is only different within species.

Sutterella- a Gram negative, rod shape, non-spore forming anaerobic bacteria that belongs to the family *Alcaligenaceae* within the phylum *Proteobacteria*.
It was found in IBS human patients, but in dogs it was inconclusive, because it was described as a bacterium maintaining healthy microbiome, and part of the healthy bacterial criteria for dysbiosis index [49] .
However it was found in AHD patients, which differs it from other and gives us new prospection in identifying the underlying bacteria that can cause canine acute enteropathies.
Sutterella plays a role in the complex ecosystem of the gastrointestinal tract. It was discovered in inflammatory bowel disease (IBD) [133], [134] and irritable bowel syndrome (IBS) in humans [135]

This difference within species may lead to a better understanding of the microbiome and different function within species.

6. Conclusions

The microbiome plays a critical role in maintaining gut health by regulating immune responses, aiding digestion, and protecting against harmful pathogens. In IBD/CE, an imbalance in the microbiome (dysbiosis) contributes to inflammation and disease progression, with altered bacterial composition and diversity affecting the gut's immune and metabolic environment. It is important to monitor and track the changes and diversity of the microbiome, because it can lead us to a future therapy option and maybe to a useful solution. It may happen that a certain bacteria or other agent can lead to dysbiosis and CE, therefore we chose to put emphasis on this topic.

This study found that beneficial bacteria such as *Faecalibacterium prausnitzii* and *Lactobacillus ruminis* were abundant in healthy dogs, while harmful bacteria like *Clostridium perfringens, Bacteroides fragilis, and Plesiomonas shigelloides* were prevalent in dogs with chronic enteropathy (CE). However, the roles of some bacteria, such as *Prevotella copri* and *Blautia producta*, remain inconclusive, as they appear to have both beneficial and harmful effects depending on the context, such as in dysbiosis or CE. These dual roles highlight the complexity of defining bacterial effects in microbiome research.

In our study, we investigated the microbiome composition of healthy and chronically sick canines from different breeds (YTE, YTCE, MBH, MBCE groups) by their fecal sample.
We also used the Acute enteropathy datasets (AHD, NHD groups), in order to see any difference between acute to chronic disease.
We used NGS (New generation sequencing) method, in order to extract the complete composition of bacteria in the 3 different groups.

Similarity within the chronic group (YTCE and MBCE) was observed only with the bacterium: ***Blautia producta***, that was in both groups without any breed differences/significance.

However, the rest of the bacteria differed within the groups:
We couldn't find an overlap with the sick Yorkshires and sick mixed breeds because of the different pattern of CE in them

We assume that because Yorkshires have PLE while other breeds tend to have another stage of CE which is not PLE in most cases.

We discovered that ***Bacteroides fragilis*** was not found in CE dogs microbiome, only in human IBD microbiome.
This bacterium was found in YTCE group, which can be a new information about a potentially harmful agents, especially from enterotoxigenic subclass that can be breed specific.

We also discovered that ***Ruminococcus gnavus*** are suspected of complications of parvo-virus enteritis. In literature review we mentioned the importance of predisposing factors such as Infectious disease such as Parvovirus. This information may lead us to a bacterium that may cause dysbiosis and complication of CE. [104]

However, ***Ruminococcus gnavus*** alone is a prominent component in the fecal microbiome of dogs with "intestinal lymphangiectasia" [123]. Lymphangiectasia is a severe complication in CE and especially PLE in Yorkshires. It was surprising to discover this bacterium in MBCE group and not in YTCE group.

With the acute enteropathy set we discovered that some bacteria have a special role with species differences.
For example, ***Turicibacter*** was inconclusive because it is mostly considered as a healthy bacterium in microbiome of both humans and dogs
However, in one human research it was abundant in IBS (Irritable bowel syndrome) in humans, while it was in lower amount in healthy patients [124]
Therefore, this bacterium remains a mystery, it is unknown yet if it is a healthy or harmful bacterium, or if its risk potential is only different within species.

Sutterella, it was found in IBS human patients, but in dogs it was inconclusive, because it was described as a bacterium maintaining healthy microbiome, and part of the healthy bacterial criteria for dysbiosis index.
However it was found in AHD patients, which differs it from other and gives us new prospection in identifying the underlying bacteria that can cause Canine enteropathies.

Our conclusion from this study highlights the profound impact of microbial dysbiosis on canine chronic enteropathies (CE), emphasizing significant reductions in beneficial taxa like *Faecalibacterium prausnitzii and Prevotella copri*, alongside an increase in pathogenic bacteria such as *Clostridium perfringens* and *Salmonella enterica*. Notably, our findings on *Bacteroides fragilis* and *Ruminococcus gnavus* suggest previously unreported, breed-specific microbiota alterations in Yorkshire Terriers, underscoring the unique microbial dynamics of protein-losing enteropathy (PLE).

Comparisons with other studies validated shared patterns of dysbiosis but revealed differences between acute and chronic conditions, such as the presence of *Turicibacter* and *Sutterella* in acute cases.

Building on prior work that links diet, stress, and microbiome health, this research further demonstrates the potential of personalized interventions, such as hydrolyzed protein diets and stress management, in restoring microbial balance and alleviating CE symptoms.

Eliminating processed food, sticking to dietary fibers, vegetables and healthy fats can assist sick individuals and can help with reducing the risk of IBD in healthy individuals, as shown in several studies. We saw that in YTCE group, once they fed on Hydrolyzed protein diet, they started to recover from dysbiosis and some of the CE symptoms. This is an important conclusion, because it shows the powerful effect of nutrition on CE [24], [25].

Stress can be a factor leading to CE [26], and it can be reduced with the right approach, such as: giving enough space to the animal at home, isolating it from intense sounds, and reducing the workload from it [27], especially in military and police dogs, that work constantly under pressure.

In our opinion, it is important to keep investigating this topic by using gene sequencing of the microbiome of many sick individuals as possible, testing sick individuals with specific types of diets, reducing “western” diet, processed food and exigent lifestyle in both sick and healthy individuals [24], [25].

A future call for better microbiome can be executed as adjustable nutrition as dry and wet food for canine, using complexed polysaccharides from un-processed cereals and legumes, the use

of vegetables and high-quality meat or hydrolyzed protein that is easier to digest, with an addition of healthy fats, especially omega 3.

Omega 3 has an anti-inflammatory effect that can aid in IBD and CE, as shown in several studies [139], [140].

In my humble opinion, it is important to investigate the topic of IBD and CE diseases further, for a better future for our animals (and humans as well).

7. Bibliography

1. Coskun M. Intestinal epithelium in inflammatory bowel disease. Frontiers in medicine. 2014;1. http://dx.doi.org/10.3389/fmed.2014.00024. https://doi.org/10.3389/fmed.2014.00024

2. Heilmann RM, Suchodolski JS. Is inflammatory bowel disease in dogs and cats associated with a Th1 or Th2 polarization? Veterinary immunology and immunopathology. 2015;168(3–4):131–134. https://linkinghub.elsevier.com/retrieve/pii/S0165242715300088. https://doi.org/10.1016/j.vetimm.2015.10.008

3. Jergens AE, Heilmann RM. Canine chronic enteropathy—Current state-of-the-art and emerging concepts. Frontiers in veterinary science. 2022;9. http://dx.doi.org/10.3389/fvets.2022.923013. https://doi.org/10.3389/fvets.2022.923013

4. Sattasathuchana P, Steiner JM. Canine eosinophilic gastrointestinal disorders. Animal health research reviews. 2014 [accessed 2024 Nov 27];15(1):76–86. https://www.cambridge.org/core/journals/animal-health-research-reviews/article/abs/canine-eosinophilic-gastrointestinal-disorders/F628D55BA6A681301A184698DAD68048. https://doi.org/10.1017/s1466252314000012

5. Umar SB, DiBaise JK. Protein-losing enteropathy: Case illustrations and clinical review. The American journal of gastroenterology. 2010;105(1):43–49. http://dx.doi.org/10.1038/ajg.2009.561. https://doi.org/10.1038/ajg.2009.561

6. Östensson M et al. Epidemiology, validation, and clinical characteristics of inflammatory bowel disease: the ABIS birth cohort study. BMC gastroenterology. 2023;23(1). http://dx.doi.org/10.1186/s12876-023-02840-1. https://doi.org/10.1186/s12876-023-02840-1

7. Halfvarson J et al. Dynamics of the human gut microbiome in inflammatory bowel disease. Nature microbiology. 2017 [accessed 2024 Nov 27];2(5):1–7. https://www.nature.com/articles/nmicrobiol20174. https://doi.org/10.1038/nmicrobiol.2017.4

8. Lucendo AJ, Rezende LCD. Importance of nutrition in inflammatory bowel disease. World journal of gastroenterology: WJG. 2009;15(17):2081. http://dx.doi.org/10.3748/wjg.15.2081. https://doi.org/10.3748/wjg.15.2081

9. Maunder RG. Evidence that stress contributes to inflammatory bowel disease: Evaluation, synthesis, and future directions. Inflammatory bowel diseases. 2005 [accessed 2024 Nov 27];11(6):600–608. https://academic.oup.com/ibdjournal/article-abstract/11/6/600/4683997. https://doi.org/10.1097/01.mib.0000161919.42878.a0

10. Bernstein CN, Shanahan F. Disorders of a modern lifestyle: reconciling the epidemiology of inflammatory bowel diseases. Gut. 2008;57(9):1185–1191. http://dx.doi.org/10.1136/gut.2007.122143. https://doi.org/10.1136/gut.2007.122143

11. Bianco AM. Genetics of inflammatory bowel disease from multifactorial to monogenic forms. World journal of gastroenterology: WJG. 2015 [accessed 2024 Nov 27];21(43):12296. https://www.wjgnet.com/1007-9327/full/v21/i43/12296.htm. https://doi.org/10.3748/wjg.v21.i43.12296

12. Ianiro G, Tilg H, Gasbarrini A. Antibiotics as deep modulators of gut microbiota: between good and evil. Gut. 2016 [accessed 2024 Nov 27];65(11):1906–1915. https://gut.bmj.com/content/65/11/1906.short. https://doi.org/10.1136/gutjnl-2016-312297

13. Petschow B et al. Probiotics, prebiotics, and the host microbiome: the science of translation. Annals of the New York Academy of Sciences. 2013;1306(1):1–17. http://dx.doi.org/10.1111/nyas.12303. https://doi.org/10.1111/nyas.12303

14. Rastogi S, Singh A. Gut microbiome and human health: Exploring how the probiotic genus Lactobacillus modulate immune responses. Frontiers in pharmacology. 2022;13. http://dx.doi.org/10.3389/fphar.2022.1042189. https://doi.org/10.3389/fphar.2022.1042189

15. Cheng J, Hu J, Geng F, Nie S. Bacteroides utilization for dietary polysaccharides and their beneficial effects on gut health. Food science and human wellness. 2022;11(5):1101–1110. https://linkinghub.elsevier.com/retrieve/pii/S2213453022000404. https://doi.org/10.1016/j.fshw.2022.04.002

16. Wang J-W et al. Fecal microbiota transplantation: Review and update. Taiwan yi zhi [Journal of the Formosan Medical Association]. 2019;118:S23–S31. https://linkinghub.elsevier.com/retrieve/pii/S0929664618305552. https://doi.org/10.1016/j.jfma.2018.08.011

17. Colman RJ, Rubin DT. Fecal microbiota transplantation as therapy for inflammatory bowel disease: A systematic review and meta-analysis. Journal of Crohn's & colitis. 2014 [accessed 2024 Nov 27];8(12):1569–1581. https://academic.oup.com/ecco-jcc/article/8/12/1569/2756228. https://doi.org/10.1016/j.crohns.2014.08.006

18. Chaitman J, Gaschen F. Fecal Microbiota transplantation in dogs. The Veterinary clinics of North America. Small animal practice. 2021;51(1):219–233. https://linkinghub.elsevier.com/retrieve/pii/S0195561620301121. https://doi.org/10.1016/j.cvsm.2020.09.012

19. Febbo E. Faecal microbiota transplantation in 16 dogs with idiopathic inflammatory bowel disease - Veterinaria. Scivac.org. 2017 Mar 2 [accessed 2024 Nov 27]. https://veterinaria.scivac.org/2017/year-31-n-1-february-2017/faecal-microbiota-transplantation-in-16-dogs-with-idiopathic-inflammatory-bowel-disease.html

20. Nakase H et al. Evidence-based clinical practice guidelines for inflammatory bowel disease 2020. Journal of gastroenterology. 2021;56(6):489–526. http://dx.doi.org/10.1007/s00535-021-01784-1. https://doi.org/10.1007/s00535-021-01784-1

21. Hemida M et al. Early life modifiable exposures and their association with owner reported inflammatory bowel disease symptoms in adult dogs. Frontiers in veterinary science. 2021;8. http://dx.doi.org/10.3389/fvets.2021.552350. https://doi.org/10.3389/fvets.2021.552350

22. Dupouy-Manescau N et al. Updating the classification of chronic inflammatory enteropathies in dogs. Animals: an open access journal from MDPI. 2024 [accessed 2024 Nov 27];14(5):681. https://www.mdpi.com/2076-2615/14/5/681. https://doi.org/10.3390/ani14050681

23. Candellone A et al. Retrospective study of 222 dogs suffering from food-responsive enteropathy—correlation with clinical variables, diet and breed. Veterinary sciences. 2024 [accessed 2024 Nov 27];11(7):294. https://www.mdpi.com/2306-7381/11/7/294. https://doi.org/10.3390/vetsci11070294

24. Drake I et al. A Western dietary pattern is prospectively associated with cardio-metabolic traits and incidence of the metabolic syndrome. The British journal of nutrition. 2018 [accessed 2024 Nov 27];119(10):1168–1176. https://www.cambridge.org/core/journals/british-

journal-of-nutrition/article/western-dietary-pattern-is-prospectively-associated-with-cardiometabolic-traits-and-incidence-of-the-metabolic-syndrome/27E9CD3FE973847C9EFC120C2CAFFA4B. https://doi.org/10.1017/s000711451800079x

25. Jergens AE, Parvinroo S, Kopper J, Wannemuehler MJ. Rules of engagement: Epithelial-microbe interactions and inflammatory bowel disease. Frontiers in medicine. 2021;8. http://dx.doi.org/10.3389/fmed.2021.669913. https://doi.org/10.3389/fmed.2021.669913

26. Kiełbik P, Witkowska-Piłaszewicz O. The relationship between canine behavioral disorders and gut microbiome and future therapeutic perspectives. Animals: an open access journal from MDPI. 2024 [accessed 2024 Nov 27];14(14):2048. https://www.mdpi.com/2076-2615/14/14/2048. https://doi.org/10.3390/ani14142048

27. Díaz-Regañón D et al. Assessing the quality of life of dogs with inflammatory bowel disease and their owners. Veterinary sciences. 2023 [accessed 2024 Nov 27];10(7):405. https://www.mdpi.com/2306-7381/10/7/405. https://doi.org/10.3390/vetsci10070405

28. Allenspach K, Mochel JP. Genetics and immunopathogenesis of chronic inflammatory enteropathies in dogs. Advances in small animal care. 2020;1:91–100. https://linkinghub.elsevier.com/retrieve/pii/S2666450X20300079. https://doi.org/10.1016/j.yasa.2020.07.007

29. Dandrieux JRS, Mansfield CS. Chronic enteropathy in canines: Prevalence, impact and management strategies. Veterinary medicine (Auckland, N.Z.). 2019;10:203–214. http://dx.doi.org/10.2147/vmrr.s162774. https://doi.org/10.2147/vmrr.s162774

30. Makielski K, Cullen J, O'Connor A, Jergens AE. Narrative review of therapies for chronic enteropathies in dogs and cats. Journal of veterinary internal medicine. 2019;33(1):11–22. http://dx.doi.org/10.1111/jvim.15345. https://doi.org/10.1111/jvim.15345

31. Kilian E et al. Long-term effects of canine parvovirus infection in dogs. PloS one. 2018;13(3):e0192198. http://dx.doi.org/10.1371/journal.pone.0192198. https://doi.org/10.1371/journal.pone.0192198

32. Sato-Takada K, Flemming AM, Voordouw MJ, Carr AP. Parvovirus enteritis and other risk factors associated with persistent gastrointestinal signs in dogs later in life: a retrospective cohort study. BMC veterinary research. 2022;18(1). http://dx.doi.org/10.1186/s12917-022-03187-7. https://doi.org/10.1186/s12917-022-03187-7

33. Šlapeta J et al. Differences in the faecal microbiome of non-diarrhoeic clinically healthy dogs and cats associated with Giardia duodenalis infection: impact of hookworms and coccidia. International journal for parasitology. 2015;45(9–10):585–594. https://linkinghub.elsevier.com/retrieve/pii/S0020751915001113. https://doi.org/10.1016/j.ijpara.2015.04.001

34. Boucard A-S et al. Age and Giardia intestinalis Infection Impact Canine Gut Microbiota. Microorganisms. 2021 [accessed 2024 Nov 27];9(9):1862. https://www.mdpi.com/2076-2607/9/9/1862. https://doi.org/10.3390/microorganisms9091862

35. Tal S et al. Developmental intestinal microbiome alterations in canine fading puppy syndrome: a prospective observational study. npj biofilms and microbiomes. 2021 [accessed 2024 Nov 27];7(1):1–10. https://www.nature.com/articles/s41522-021-00222-7. https://doi.org/10.1038/s41522-021-00222-7

36. Masuoka H et al. Transition of the intestinal microbiota of dogs with age. Bioscience of microbiota, food and health. 2017;36(1):27–31. https://www.jstage.jst.go.jp/article/bmfh/36/1/36_BMFH-2016-021/_article. https://doi.org/10.12938/bmfh.bmfh-2016-021

37. Goddard A, Leisewitz AL. Canine Parvovirus. The Veterinary clinics of North America. Small animal practice. 2010;40(6):1041–1053. https://linkinghub.elsevier.com/retrieve/pii/S019556161000094X. https://doi.org/10.1016/j.cvsm.2010.07.007

38. Park JS et al. Intestinal microbial dysbiosis in beagles naturally infected with canine Parvovirus. Journal of microbiology and biotechnology. 2019;29(9):1391–1400. http://dx.doi.org/10.4014/jmb.1901.01047. https://doi.org/10.4014/jmb.1901.01047

39. Simmerson SM et al. Clinical features, intestinal histopathology, and outcome in protein-losing enteropathy in Yorkshire Terrier dogs. Journal of veterinary internal medicine. 2014;28(2):331–337. http://dx.doi.org/10.1111/jvim.12291. https://doi.org/10.1111/jvim.12291

40. Craven MD, Washabau RJ. Comparative pathophysiology and management of protein-losing enteropathy. Journal of veterinary internal medicine. 2019;33(2):383–402. http://dx.doi.org/10.1111/jvim.15406. https://doi.org/10.1111/jvim.15406

41. Garrigues Q, Apper E, Chastant S, Mila H. Gut microbiota development in the growing dog: A dynamic process influenced by maternal, environmental and host factors. Frontiers in veterinary science. 2022;9. http://dx.doi.org/10.3389/fvets.2022.964649. https://doi.org/10.3389/fvets.2022.964649

42. Zakošek Pipan M et al. Do newborn puppies have their own microbiota at birth? Influence of type of birth on newborn puppy microbiota. Theriogenology. 2020;152:18–28. https://linkinghub.elsevier.com/retrieve/pii/S0093691X2030234X. https://doi.org/10.1016/j.theriogenology.2020.04.014

43. Pilla R, Suchodolski JS. The role of the canine gut microbiome and metabolome in health and gastrointestinal disease. Frontiers in veterinary science. 2020;6. http://dx.doi.org/10.3389/fvets.2019.00498. https://doi.org/10.3389/fvets.2019.00498

44. Liao P et al. Abrupt dietary change and gradual dietary transition impact diarrheal symptoms, fecal fermentation characteristics, Microbiota, and metabolic profile in healthy puppies. Animals: an open access journal from MDPI. 2023 [accessed 2024 Nov 27];13(8):1300. https://www.mdpi.com/2076-2615/13/8/1300. https://doi.org/10.3390/ani13081300

45. Vuori KA et al. The effect of puppyhood and adolescent diet on the incidence of chronic enteropathy in dogs later in life. Scientific reports. 2023 [accessed 2024 Nov 27];13(1):1–14. https://www.nature.com/articles/s41598-023-27866-z. https://doi.org/10.1038/s41598-023-27866-z

46. Petersen C, Round JL. Defining dysbiosis and its influence on host immunity and disease. Cellular microbiology. 2014;16(7):1024–1033. http://dx.doi.org/10.1111/cmi.12308. https://doi.org/10.1111/cmi.12308

47. Tamboli CP, Neut C, Desreumaux P, Colombel JF. Dysbiosis as a prerequisite for IBD. Gut. 2004 [accessed 2024 Nov 27];53(7):1057. https://gut.bmj.com/content/53/7/1057.1#linked-articles

48. Suchodolski JS. Diagnosis and interpretation of intestinal dysbiosis in dogs and cats. Veterinary journal (London, England: 1997). 2016;215:30–37. https://linkinghub.elsevier.com/retrieve/pii/S1090023316300338. https://doi.org/10.1016/j.tvjl.2016.04.011

49. Canine and feline Microbiota dysbiosis index. Gastrointestinal Laboratory. 2020 Jul 30 [accessed 2024 Nov 27]. https://vetmed.tamu.edu/gilab/service/assays/canine-microbiota-dysbiosis-index/

50. AlShawaqfeh MK et al. A dysbiosis index to assess microbial changes in fecal samples of dogs with chronic inflammatory enteropathy. FEMS microbiology ecology. 2017 [accessed 2024 Nov 27];93(11):fix136. https://academic.oup.com/femsec/article/93/11/fix136/4443197?login=false. https://doi.org/10.1093/femsec/fix136

51. Chauhan T. What is Metagenomics?- definition, steps, process and applications. Genetic Education. 2020 Aug 10 [accessed 2024 Nov 27]. https://geneticeducation.co.in/what-is-metagenomics-definition-steps-process-and-applications/

52. Hu T, Chitnis N, Monos D, Dinh A. Next-generation sequencing technologies: An overview. Human immunology. 2021;82(11):801–811. https://linkinghub.elsevier.com/retrieve/pii/S0198885921000628. https://doi.org/10.1016/j.humimm.2021.02.012

53. Laudadio I et al. Quantitative assessment of shotgun metagenomics and 16S rDNA amplicon sequencing in the study of human gut microbiome. Omics: a journal of integrative biology. 2018;22(4):248–254. http://dx.doi.org/10.1089/omi.2018.0013. https://doi.org/10.1089/omi.2018.0013

54. Malla MA et al. Exploring the human microbiome: The potential future role of next-generation sequencing in disease diagnosis and treatment. Frontiers in immunology. 2019;9. http://dx.doi.org/10.3389/fimmu.2018.02868. https://doi.org/10.3389/fimmu.2018.0286

55. Janda JM, Abbott SL. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. Journal of clinical microbiology. 2007;45(9):2761–2764. http://dx.doi.org/10.1128/jcm.01228-07. https://doi.org/10.1128/jcm.01228-07

56. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120 (2014).

57. Wood, D. E., Lu, J. & Langmead, B. Improved metagenomic analysis with Kraken2. Genome biology 20, 257 (2019).

58. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria (2023).

59. McMurdie, P. J. & Holmes, S. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLOS ONE 8, 1–11 (2013).

60. Lahti, L. & Shetty, S. microbiome R package (2012-2019).

61. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550 (2014).

62. Díaz-Regañón D et al. Characterization of the fecal and mucosa-associated Microbiota in dogs with chronic inflammatory enteropathy. Animals: an open access journal from MDPI. 2023 [accessed 2024 Nov 27];13(3):326. https://www.mdpi.com/2076-2615/13/3/326. https://doi.org/10.3390/ani13030326

63. Doulidis PG et al. Gut microbiome signatures of Yorkshire Terrier enteropathy during disease and remission. Scientific reports. 2023 [accessed 2024 Nov 27];13(1):1–11. https://www.nature.com/articles/s41598-023-31024-w. https://doi.org/10.1038/s41598-023-31024-w

64. Gut metagenome of canine with acute hemorrhagic diarrhea targeting the... - SRA - NCBI. Nih.gov. [accessed 2024 Nov 27]. https://www.ncbi.nlm.nih.gov/sra/SRX445842%5baccn%5d

65. Raw sequence reads for canine IBD - SRA - NCBI. Nih.gov. [accessed 2024 Nov 27]. https://www.ncbi.nlm.nih.gov/sra/SRX3190251[accn]

66. Gut metagenome of canine with inflammatory bowel disease pre-treatment... - SRA - NCBI. Nih.gov. [accessed 2024 Nov 27]. https://www.ncbi.nlm.nih.gov/sra/SRX495869[accn]

67. Ural K, Erdogan H, Erdogan S, Balikci C. Gut microbiota and relevant abundances of *Prevotella copri, Lachnospiraceae, Collinsella, Helicobacter cinaedi, Desulfovibrio*, and *Escherichia coli* among cats with *Pemphigus foliaceus*. Ethiopian veterinary journal. 2024 [accessed 2024 Nov 27];28(1):105–121. https://www.ajol.info/index.php/evj/article/view/267099. https://doi.org/10.4314/evj.v28i1.7

68. Dahal RH et al. Insight into gut dysbiosis of patients with inflammatory bowel disease and ischemic colitis. Frontiers in microbiology. 2023;14. http://dx.doi.org/10.3389/fmicb.2023.1174832. https://doi.org/10.3389/fmicb.2023.1174832

69. Wang X, Cao H. P090 Prevotella copri promotes colitis in mice by reducing expression of ATF4 and disturbing the gut microbiota. Journal of Crohn's & colitis. 2024 [accessed 2024 Nov 27];18(Supplement_1):i368–i368. https://academic.oup.com/ecco-jcc/article/18/Supplement_1/i368/7586366. https://doi.org/10.1093/ecco-jcc/jjad212.0220

70. Alpizar-Rodriguez D et al. *Prevotella copri* in individuals at risk for rheumatoid arthritis. Annals of the rheumatic diseases. 2019 [accessed 2024 Nov 27];78(5):590–593. https://ard.bmj.com/content/78/5/590.abstract. https://doi.org/10.1136/annrheumdis-2018-214514

71. Hernandez J et al. Domestic environment and gut Microbiota: Lessons from pet dogs. Microorganisms. 2022 [accessed 2024 Nov 27];10(5):949. https://www.mdpi.com/2076-2607/10/5/949. https://doi.org/10.3390/microorganisms10050949

72. Mukherjee A, Lordan C, Ross RP, Cotter PD. Gut microbes from the phylogenetically diverse genus*Eubacterium*and their various contributions to gut health. Gut microbes. 2020;12(1):1802866. http://dx.doi.org/10.1080/19490976.2020.1802866. https://doi.org/10.1080/19490976.2020.1802866

73. Beloshapka AN et al. Fecal microbial communities of healthy adult dogs fed raw meat-based diets with or without inulin or yeast cell wall extracts as assessed by 454 pyrosequencing. FEMS microbiology ecology. 2013 [accessed 2024 Nov 27];84(3):532–541.

https://academic.oup.com/femsec/article/84/3/532/579061. https://doi.org/10.1111/1574-6941.12081

74. Maldonado-Contreras A et al. Dysbiosis in a canine model of human fistulizing Crohn's disease. Gut microbes. 2020;12(1):1785246. http://dx.doi.org/10.1080/19490976.2020.1785246. https://doi.org/10.1080/19490976.2020.1785246

75. Wexler AG, Goodman AL. An insider's perspective: Bacteroides as a window into the microbiome. Nature microbiology. 2017 [accessed 2024 Nov 27];2(5):1–11. https://www.nature.com/articles/nmicrobiol201726. https://doi.org/10.1038/nmicrobiol.2017.26

76. Matsuoka K. Fecal microbiota transplantation for ulcerative colitis. Immunological medicine. 2021;44(1):30–34. http://dx.doi.org/10.1080/25785826.2020.1792040. https://doi.org/10.1080/25785826.2020.1792040

77. Sung C-H et al. Dysbiosis index to evaluate the fecal microbiota in healthy cats and cats with chronic enteropathies. Journal of feline medicine and surgery. 2022;24(6):e1–e12. http://dx.doi.org/10.1177/1098612x221077876. https://doi.org/10.1177/1098612x221077876

78. Akama K, Otani S. Clostridium perfringens as the flora in the intestine of healthy persons. Japanese journal of medical science & biology. 1970;23(3):161–175. https://www.jstage.jst.go.jp/article/yoken1952/23/3/23_3_161/_article. https://doi.org/10.7883/yoken1952.23.161

79. Kiu R, Hall LJ. An update on the human and animal enteric pathogen *Clostridium perfringens*. Emerging microbes & infections. 2018;7(1):1–15. http://dx.doi.org/10.1038/s41426-018-0144-8. https://doi.org/10.1038/s41426-018-0144-8

80. Silva ROS, Lobato FCF. Clostridium perfringens: A review of enteric diseases in dogs, cats and wild animals. Anaerobe. 2015;33:14–17. https://linkinghub.elsevier.com/retrieve/pii/S1075996415000074. https://doi.org/10.1016/j.anaerobe.2015.01.006

81. Rood JI et al. Expansion of the Clostridium perfringens toxin-based typing scheme. Anaerobe. 2018;53:5–10. https://linkinghub.elsevier.com/retrieve/pii/S1075996418300684. https://doi.org/10.1016/j.anaerobe.2018.04.011

82. Ziese A-L et al. Correction: Effect of probiotic treatment on the clinical course, intestinal microbiome, and toxigenic Clostridium perfringens in dogs with acute hemorrhagic diarrhea. PloS one. 2023;18(1):e0280539. http://dx.doi.org/10.1371/journal.pone.0280539. https://doi.org/10.1371/journal.pone.0280539

83. Hanifeh M et al. Adhesion of Bacteroides vulgatus and Fusobacterium varium to the Colonic Mucosa of Healthy Beagles. Veterinary sciences. 2024 [accessed 2024 Nov 27];11(7):319. https://www.mdpi.com/2306-7381/11/7/319. https://doi.org/10.3390/vetsci11070319

84. Carrow HC, Batachari LE, Chu H. Strain diversity in the microbiome: Lessons from Bacteroides fragilis. PLoS pathogens. 2020;16(12):e1009056. http://dx.doi.org/10.1371/journal.ppat.1009056. https://doi.org/10.1371/journal.ppat.1009056

85. Rabizadeh S et al. Enterotoxigenic Bacteroides fragilis: A potential instigator of colitis. Inflammatory bowel diseases. 2007 [accessed 2024 Nov 27];13(12):1475–1483. https://academic.oup.com/ibdjournal/article-abstract/13/12/1475/4652990. https://doi.org/10.1002/ibd.20265

86. Zamani S et al. Detection of enterotoxigenic Bacteroides fragilis in patients with ulcerative colitis. Gut pathogens. 2017;9(1). http://dx.doi.org/10.1186/s13099-017-0202-0. https://doi.org/10.1186/s13099-017-0202-0

87. Reis, A. C. M., Silva, J. O., Laranjeira, B. J., Pinheiro, A. Q., & Carvalho, C. B. M. (2014). Virulence factors and biofilm production by isolates of Bacteroides fragilis recovered from dog intestinal tracts. *Brazilian Journal of Microbiology*, *45*(2), 647–650. https://doi.org/10.1590/s1517-83822014000200037

88. Loomis KH et al. A mixed community of skin microbiome representatives influences cutaneous processes more than individual members. Microbiome. 2021;9(1). http://dx.doi.org/10.1186/s40168-020-00963-1. https://doi.org/10.1186/s40168-020-00963-1

89. Zhao Y, Jiang Q. Roles of the polyphenol–gut Microbiota interaction in alleviating colitis and preventing colitis-associated colorectal cancer. Advances in nutrition (Bethesda, Md.). 2021;12(2):546–565. http://academic.oup.com/advances/article-pdf/12/2/546/38878360/nmaa104.pdf. https://doi.org/10.1093/advances/nmaa104

90. Carapeto S et al. Effect of the administration of a lyophilised faecal capsules on the intestinal microbiome of dogs: A pilot study. Genes. 2023 [accessed 2024 Nov 27];14(9):1676. https://www.mdpi.com/2073-4425/14/9/1676. https://doi.org/10.3390/genes14091676

91. Rinkinen ML et al. Streptococcus alactolyticus is the dominating culturable lactic acid bacterium species in canine jejunum and feces of four fistulated dogs. FEMS microbiology letters. 2004 [accessed 2024 Nov 27];230(1):35–39. https://academic.oup.com/femsle/article-abstract/230/1/35/765194?redirectedFrom=fulltext. https://doi.org/10.1016/s0378-1097(03)00851-6

92. Bajer L et al. Distinct gut microbiota profiles in patients with primary sclerosing cholangitis and ulcerative colitis. World journal of gastroenterology: WJG. 2017 [accessed 2024 Nov 28];23(25):4548. https://www.wjgnet.com/1007-9327/full/v23/i25/4548.htm. https://doi.org/10.3748/wjg.v23.i25.4548

93. Prins FM et al. The gut microbiome across the cardiovascular risk spectrum. European journal of preventive cardiology. 2024 [accessed 2024 Nov 28];31(8):935–944. https://academic.oup.com/eurjpc/article/31/8/935/7462174. https://doi.org/10.1093/eurjpc/zwad377

94. Xu H et al. Oral administration of compound probiotics improved canine feed intake, weight gain, immunity and intestinal Microbiota. Frontiers in immunology. 2019;10. http://dx.doi.org/10.3389/fimmu.2019.00666. https://doi.org/10.3389/fimmu.2019.00666

95. Wu C et al. Strain-level screening of human gut microbes identifies Blautia producta as a novel anti-hyperlipidemic probiotic via the production of 12-methylmyristic acid. Research Square. 2021. http://dx.doi.org/10.21203/rs.3.rs-989302/v1. https://doi.org/10.21203/rs.3.rs-989302/v1

96. Maturana JL, Cárdenas JP. Insights on the evolutionary genomics of the Blautia genus: Potential new species and genetic content among lineages. Frontiers in microbiology. 2021;12. http://dx.doi.org/10.3389/fmicb.2021.660920. https://doi.org/10.3389/fmicb.2021.660920

97. Sokol H et al. Specificities of the intestinal microbiota in patients with inflammatory bowel disease and *Clostridium difficile* infection. Gut microbes. 2018;9(1):55–60. http://dx.doi.org/10.1080/19490976.2017.1361092. https://doi.org/10.1080/19490976.2017.1361092

98. Benítez-Páez A et al. Depletion of Blautia species in the Microbiota of obese children relates to intestinal inflammation and metabolic phenotype worsening. mSystems. 2020;5(2). http://dx.doi.org/10.1128/mSystems.00857-19. https://doi.org/10.1128/mSystems.00857-19

99. Davrandi M et al. The relationship between mucosal Microbiota, colitis, and systemic inflammation in chronic granulomatous disorder. Journal of clinical immunology. 2022;42(2):312–324. http://dx.doi.org/10.1007/s10875-021-01165-6. https://doi.org/10.1007/s10875-021-01165-6

100. Li Q et al. Gut dysbiosis and its associations with gut Microbiota-derived metabolites in dogs with myxomatous mitral valve disease. mSystems. 2021;6(2). http://dx.doi.org/10.1128/msystems.00111-21. https://doi.org/10.1128/msystems.00111-21

101. Parsaei M, Sarafraz N, Moaddab SY, Ebrahimzadeh Leylabadlo H. The importance of Faecalibacterium prausnitzii in human health and diseases. New microbes and new infections. 2021;43(100928):100928. https://linkinghub.elsevier.com/retrieve/pii/S2052297521000925. https://doi.org/10.1016/j.nmni.2021.100928

102. Huang Z et al. The canine gastrointestinal microbiota: early studies and research frontiers. Gut microbes. 2020;11(4):635–654. http://dx.doi.org/10.1080/19490976.2019.1704142. https://doi.org/10.1080/19490976.2019.1704142

103. Miquel S et al. Faecalibacterium prausnitzii and human intestinal health. Current opinion in microbiology. 2013;16(3):255–261. https://linkinghub.elsevier.com/retrieve/pii/S1369527413000775. https://doi.org/10.1016/j.mib.2013.06.003

104. Pessione E. Lactic acid bacteria contribution to gut microbiota complexity: lights and shadows. Frontiers in cellular and infection microbiology. 2012;2. http://dx.doi.org/10.3389/fcimb.2012.00086. https://doi.org/10.3389/fcimb.2012.00086

105. O' Donnell MM et al. Lactobacillus ruminis strains cluster according to their mammalian gut source. BMC microbiology. 2015;15(1). http://dx.doi.org/10.1186/s12866-015-0403-y. https://doi.org/10.1186/s12866-015-0403-y

106. Forde BM et al. Genome sequences and comparative genomics of two Lactobacillus ruminis strains from the bovine and human intestinal tracts. Microbial cell factories. 2011;10(S1). http://dx.doi.org/10.1186/1475-2859-10-s1-s13. https://doi.org/10.1186/1475-2859-10-s1-s13

107. Taweechotipatr M et al. *Lactobacillus saerimneri*and*Lactobacillus ruminis*: novel human-derived probiotic strains with immunomodulatory activities. FEMS microbiology letters. 2009 [accessed 2024 Nov 28];293(1):65–72. https://academic.oup.com/femsle/article-abstract/293/1/65/498454. https://doi.org/10.1111/j.1574-6968.2009.01506.x

108. Mu Q, Tavella VJ, Luo XM. Role of Lactobacillus reuteri in Human Health and Diseases. Frontiers in microbiology. 2018;9. http://dx.doi.org/10.3389/fmicb.2018.00757. https://doi.org/10.3389/fmicb.2018.00757

109. Coman MM et al. Probiotic characterization of *Lactobacillus* isolates from canine faeces. Journal of applied microbiology. 2019 [accessed 2024 Nov 28];126(4):1245–1256. https://academic.oup.com/jambio/article-abstract/126/4/1245/6714756. https://doi.org/10.1111/jam.14197

110. Hou C et al. Study and use of the probiotic Lactobacillus reuteri in pigs: a review. Journal of animal science and biotechnology. 2015;6(1). http://dx.doi.org/10.1186/s40104-015-0014-3. https://doi.org/10.1186/s40104-015-0014-3

111. Arai T et al. A survey of*Plesiomonas shigelloides*from aquatic environments, domestic animals, pets and humans. The journal of hygiene. 1980 [accessed 2024 Nov 28];84(2):203–211. https://www.cambridge.org/core/journals/epidemiology-and-infection/article/survey-of-plesiomonas-shigelloides-from-aquatic-environments-domestic-animals-pets-and-

humans/1F59D11BBEC0B516D8417C0F01555BC7.
https://doi.org/10.1017/s002217240002670x

112. Kaiser L, Surawicz CM. Infectious causes of chronic diarrhoea. Best practice & research. Clinical gastroenterology. 2012;26(5):563–571.
https://linkinghub.elsevier.com/retrieve/pii/S152169181200100X.
https://doi.org/10.1016/j.bpg.2012.11.001

113. GonzÃ¡lez-Rey C et al. Specific detection of*Plesiomonas shigelloides*isolated from aquatic environments, animals and human diarrhoeal cases by PCR based on 23S rRNA gene. FEMS immunology and medical microbiology. 2000 [accessed 2024 Nov 28];29(2):107–113. https://academic.oup.com/femspd/article/29/2/107/518344. https://doi.org/10.1111/j.1574-695x.2000.tb01512.x

114. González-Rey C et al. Molecular evidence of Plesiomonas shigelloides as a possible zoonotic agent. Folia microbiologica. 2011;56(2):178–184. http://dx.doi.org/10.1007/s12223-011-0032-2. https://doi.org/10.1007/s12223-011-0032-2

115. Rogers AWL, Tsolis RM, Bäumler AJ. *salmonella* versus the microbiome. Microbiology and molecular biology reviews: MMBR. 2021;85(1). http://dx.doi.org/10.1128/mmbr.00027-19. https://doi.org/10.1128/mmbr.00027-19

116. Usmael B et al. Isolation, antimicrobial susceptibility patterns, and risk factors assessment of non-typhoidal Salmonella from apparently healthy and diarrheic dogs. BMC veterinary research. 2022;18(1). http://dx.doi.org/10.1186/s12917-021-03135-x.
https://doi.org/10.1186/s12917-021-03135-x

117. Tripathi MK et al. Ulcerative colitis and its association with*salmonella*species. Interdisciplinary perspectives on infectious diseases. 2016;2016:1–7.
http://dx.doi.org/10.1155/2016/5854285. https://doi.org/10.1155/2016/5854285

118. Móritz AV et al. Flavonoids in mitigating the adverse effects of canine endotoxemia. Frontiers in veterinary science. 2024;11. http://dx.doi.org/10.3389/fvets.2024.1396870.
https://doi.org/10.3389/fvets.2024.1396870

119. Hejazi A, Falkiner FR. Serratia marcescens. Journal of medical microbiology. 1997;46(11):903–912. http://dx.doi.org/10.1099/00222615-46-11-903. https://doi.org/10.1099/00222615-46-11-903

120. Ochieng JB et al. *Serratia marcescens*is injurious to intestinal epithelial cells. Gut microbes. 2014;5(6):729–736. http://dx.doi.org/10.4161/19490976.2014.972223. https://doi.org/10.4161/19490976.2014.972223

121. Lobetti RG et al. Bacterial colonization of intravenous catheters in young dogs suspected to have parvoviral enteritis. Journal of the American Veterinary Medical Association. 2002 [accessed 2024 Nov 28];220(9):1321–1324. https://avmajournals.avma.org/view/journals/javma/220/9/javma.2002.220.1321.xml. https://doi.org/10.2460/javma.2002.220.1321

122. Park J, Yoon JS. Cutaneous *Serratia marcescens* infection in two dogs. The journal of small animal practice. 2024;65(4):270–273. http://dx.doi.org/10.1111/jsap.13709. https://doi.org/10.1111/jsap.13709

123. Nagahara T et al. Analysis of fecal microbial profiles in dogs with intestinal lymphangiectasia. The Journal of veterinary medical science. 2023;85(2):199–206. http://dx.doi.org/10.1292/jvms.22-0172. https://doi.org/10.1292/jvms.22-0172

124. Gryaznova M et al. Fecal Microbiota characteristics in constipation-predominant and mixed-type irritable bowel syndrome. Microorganisms. 2024 [accessed 2024 Nov 28];12(7):1414. https://www.mdpi.com/2076-2607/12/7/1414. https://doi.org/10.3390/microorganisms12071414

125. Lynch JB et al. Gut microbiota Turicibacter strains differentially modify bile acids and host lipids. Nature communications. 2023 [accessed 2024 Nov 28];14(1):1–15. https://www.nature.com/articles/s41467-023-39403-7. https://doi.org/10.1038/s41467-023-39403-7

126. Čipčić Paljetak H et al. Gut microbiota in mucosa and feces of newly diagnosed, treatment-naïve adult inflammatory bowel disease and irritable bowel syndrome patients. Gut microbes. 2022;14(1). http://dx.doi.org/10.1080/19490976.2022.2083419. https://doi.org/10.1080/19490976.2022.2083419

127. Henke MT et al. *Ruminococcus gnavus* , a member of the human gut microbiome associated with Crohn's disease, produces an inflammatory polysaccharide. Proceedings of the National Academy of Sciences of the United States of America. 2019;116(26):12672–12677. http://dx.doi.org/10.1073/pnas.1904099116. https://doi.org/10.1073/pnas.1904099116

128. Crost EH, Coletto E, Bell A, Juge N. *Ruminococcus gnavus*: friend or foe for human health. FEMS microbiology reviews. 2023 [accessed 2024 Nov 28];47(2):fuad014. https://academic.oup.com/femsre/article/47/2/fuad014/7104064. https://doi.org/10.1093/femsre/fuad014

129. Wang B, Wang X-L. Species diversity of fecal microbial flora in Canis lupus familiaris infected with canine parvovirus. Veterinary microbiology. 2019;237(108390):108390. https://linkinghub.elsevier.com/retrieve/pii/S0378113519300604. https://doi.org/10.1016/j.vetmic.2019.108390

130. Lisjak A et al. A comparison of the oral Microbiota in healthy dogs and dogs with oral tumors. Animals: an open access journal from MDPI. 2023 [accessed 2024 Nov 28];13(23):3594. https://www.mdpi.com/2076-2615/13/23/3594. https://doi.org/10.3390/ani13233594

131. Su W et al. Fusobacterium nucleatum promotes the development of ulcerative colitis by inducing the autophagic cell death of intestinal epithelial. Frontiers in cellular and infection microbiology. 2020;10. http://dx.doi.org/10.3389/fcimb.2020.594806. https://doi.org/10.3389/fcimb.2020.594806

132. Senhorinho GNA et al. Occurrence and antimicrobial susceptibility of Porphyromonas spp. and Fusobacterium spp. in dogs with and without periodontitis. Anaerobe. 2012;18(4):381–385. https://linkinghub.elsevier.com/retrieve/pii/S1075996412000480. https://doi.org/10.1016/j.anaerobe.2012.04.008

133. Hiippala K et al. Mucosal prevalence and interactions with the epithelium indicate commensalism of Sutterella spp. Frontiers in microbiology. 2016;7. http://dx.doi.org/10.3389/fmicb.2016.01706. https://doi.org/10.3389/fmicb.2016.01706

134. Mukhopadhya I et al. A comprehensive evaluation of colonic mucosal isolates of Sutterella wadsworthensis from inflammatory bowel disease. PloS one. 2011;6(10):e27076. http://dx.doi.org/10.1371/journal.pone.0027076. https://doi.org/10.1371/journal.pone.0027076

135. Zhou Y et al. Bifico relieves irritable bowel syndrome by regulating gut microbiota dysbiosis and inflammatory cytokines. European journal of nutrition. 2023;62(1):139–155. http://dx.doi.org/10.1007/s00394-022-02958-0. https://doi.org/10.1007/s00394-022-02958-0

136. Tavares-Carreon, F., De Anda-Mora, K., Rojas-Barrera, I. C., & Andrade, A. (2023). *Serratia marcescens* antibiotic resistance mechanisms of an opportunistic pathogen: a literature review. *PeerJ*, *11*(e14399), e14399. https://doi.org/10.7717/peerj.14399

137. Galler, A. I., Suchodolski, J. S., Steiner, J. M., Sung, C.-H., Hittmair, K. M., Richter, B., & Burgener, I. A. (2022). Microbial dysbiosis and fecal metabolomic perturbations in Yorkshire Terriers with chronic enteropathy. *Scientific Reports*, *12*(1), 1–17. https://doi.org/10.1038/s41598-022-17244-6

138. Pignataro, G., Di Prinzio, R., Crisi, P. E., Belà, B., Fusaro, I., Trevisan, C., De Acetis, L., & Gramenzi, A. (2021). Comparison of the therapeutic effect of treatment with antibiotics or nutraceuticals on clinical activity and the fecal microbiome of dogs with acute diarrhea. *Animals: An Open Access Journal from MDPI*, *11*(6), 1484. https://doi.org/10.3390/ani11061484

139. Ontsouka EC et al. Fish-meal diet enriched with omega-3 PUFA and treatment of canine chronic enteropathies. European journal of lipid science and technology: EJLST. 2012;114(4):412–422. http://dx.doi.org/10.1002/ejlt.201100343. https://doi.org/10.1002/ejlt.201100343

140. Lenox CE, Bauer JE. Potential adverse effects of omega-3 fatty acids in dogs and cats. Journal of veterinary internal medicine. 2013;27(2):217–226. http://dx.doi.org/10.1111/jvim.12033. https://doi.org/10.1111/jvim.12033

141. Falconi, M., & Pederzoli, P. (2001). The relevance of gastrointestinal fistulae in clinical practice: a review. *Gut*, *49*(Supplement 4), iv2–iv10. https://doi.org/10.1136/gut.49.suppl_4.iv2

142. Grześkowiak, Ł., Endo, A., Beasley, S., & Salminen, S. (2015). Microbiota and probiotics

in canine and feline welfare. *Anaerobe*, *34*, 14–23. https://doi.org/10.1016/j.anaerobe.2015.04.002

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9. Statements

9.1. Supervisor Counter-Signature Form