# The potential of the microbiome and inflammatory markers in the faeces of dogs with GI disorders

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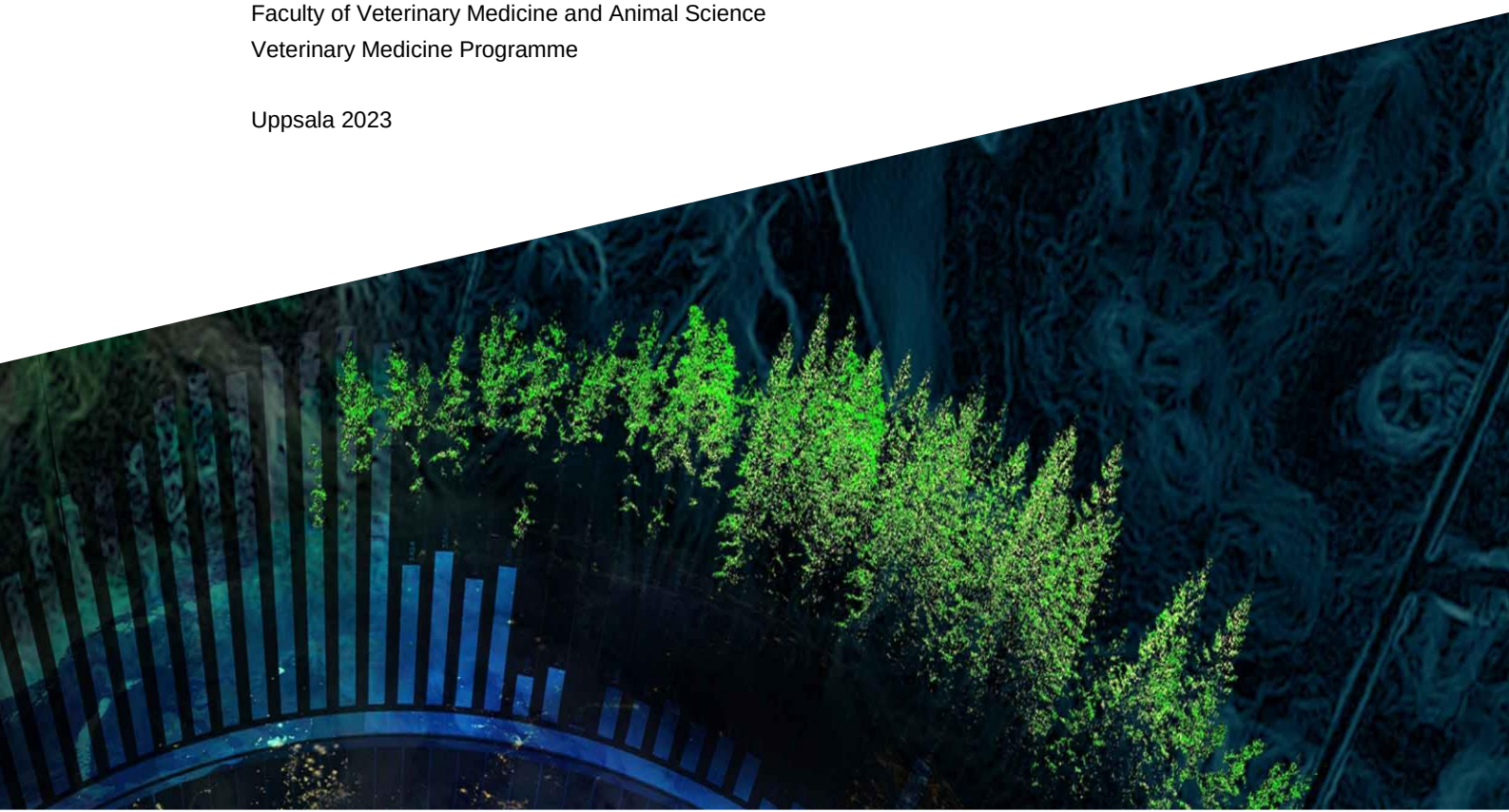

# The potential of the microbiome and inflammatory markers in the faeces of dogs with GI disorders

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## Abstract

The gut microbiota greatly affects host health. It can hinder the growth of pathogenic bacteria, decrease intestinal wall permeability, modulate the immune system, and provide nutrients for enterocytes. However, an imbalance of the gut microbiota to the point of dysbiosis can cause both acute, and in time, chronic GI disorders.

There are a wide variety of causes for GI disorders, with many different types of clinical presentations. The identification of key biomarkers for gastrointestinal functionality in dogs with GI disorders has the potential to aid the clinician as both a diagnostic tool and as a guideline for the optimal treatment plan.

This study aims to investigate the importance of the gut microbiota and calprotectin in GI disorders. 22 faecal samples have so far been collected. The owners of the dogs have answered a survey mainly focusing on general health, feed and possible co-morbidities and concomitant medications.

Of the 22 dogs sampled from, 12 were healthy controls, 2 suffered from acute gastroenteritis and 8 suffered from chronic gastrointestinal disorders. 100% of dogs who suffered from GI disorders had been treated at least once during the last year due to vomiting and/or diarrhoea in relation to 8% of the HC.

As their main diet, 90% of dogs with GI disorders were fed traditional dry and/or wet food and 10% were fed exclusively raw meat food. 67% of the HC were fed traditional dry and or wet food as their main diet, 8% were fed exclusively raw meat food and 25% were fed a combination of the above.

At a later part of this study, more samples will be collected to analyze correlations between the gut microbiota, calprotectin, and GI disorders. This study cannot as of yet provide a comprehensive review of these factors in relation to host health due to the fact that the analyses has not yet been performed. However, other studies in this field have shown that the gut microbiota and calprotectin does have the potential to improve diagnostics and prognostication of GI disease.



*Keywords:* Gut microbiota, dog, diets, calprotectin, dysbiosis, gastrointestinal disease

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# Abbreviations

| | |
|---|---|
| AHD | Acute haemorrhagic diarrhea |
| APFD | Animal protein-free diets |
| ARE | Antibiotic responsive enteropathy |
| CE | Chronic enteropathy |
| DI | Dysbiosis index |
| FRE | Food responsive enteropathy |
| GI | Gastrointestinal |
| HC | Healthy controls |
| IRE | Immunosuppressant-responsive enteropathy |
| NHD | Non-haemorrhagic diarrhea |
| SCFAs | Short-chain fatty acids |

# 1. Introduction

Gastrointestinal (GI) disease is one of the most common reasons for pet owners to visit the veterinarian (AVMA, Agria). There are many different causes of GI disease, and they can present themselves with a wide variety of non-specific clinical signs such as diarrhea, vomiting, abdominal pain, and inappetence. It is challenging for clinicians to differentiate between GI conditions using today's diagnostic tools. These tools include serology, radiography, ultrasound, elimination diets, endoscopy, and more (Willard, 2019). Several of these techniques are invasive, time-consuming, and relatively expensive.

Intestinal bacteria greatly affect host health. They act as a barrier against pathogens, help digest and harvest energy from the diet, stimulate the immune system and give nutritional support to enterocytes (Suchodolski 2011a). Studies have also implicated both specific pathogenic bacteria and dysbiosis as important factors in the pathogenesis of GI disease (Suchodolski *et al*. 2012, Vázquez-Baeza *et al.* 2016). Therefore, analyzing the microbiota can be a great inexpensive, simple, and non-invasive diagnostic tool for clinicians to differentiate between different GI disorders.

In addition to the microbiota, calprotectin, a neutrophil protein present in both plasma and feces, can also be a great diagnostic tool for GI disorders since it can indicate the severity of GI disease (Heilmann *et al.* 2018). In conclusion, increased knowledge has the potential to improve diagnostics and prognostication of the individual dog and thus improve quality of life with better clinical decision-making and individualized medicine. Both the microbiota and calprotectin can be analyzed using stool samples.

The aim of this study is to:

1. Review currently available research concerning the importance of the gut microbiota and calprotectin in GI disease.
2. Have the owners from which dogs' fecal samples are collected, answer a survey, mainly focusing on general health, feed and possible co-morbidities and concomitant medications. Results from the surveys collected are presented in the result section.

3. Initiate sampling in preparation for a later stage in the study where the aim will be to analyze any correlations between the microbiome and/or calprotectin and GI disorders, as described in the materials and methods section.

The data collected from normal controls in this study will also later serve as a reference group for another ongoing study analyzing the same parameters from dogs undergoing cytostatic treatment.

# 2. Literature Review

## 2.1 The canine gut microbiota

The microbial profile varies from dog to dog, mainly on bacterial species and strain level. The microbiome also differs inside the different compartments of the gastrointestinal tract (Suchodolski 2011a). The quantity of bacteria and their diversity increases along the GI tract and can also vary from the lumen and the mucosa. The microbial communities vary along the GI tract and reflect their microenvironment, there are for example aerobe and facultative anaerobe bacteria in the small intestine and almost exclusively anaerobe bacteria in the colon (Pilla & Suchodolski 2020).

More than 99% of bacterial phyla in the gut microbiota are comprised of Bacteroidetes, Fusobacteria, Firmicutes, Proteobacteria, and Actinobacteria (Suchodolski 2011a, Suchodolski 2011b). The gastrointestinal tract is estimated to be inhabited by $10^{12}$ to $10^{14}$ microbials, which is around 10 times the number of host cells.

### 2.1.1 The gut microbiota and Short-chain fatty acids (SCFAs)

Some bacteria in the digestive tract produce SCFAs by fermentation of non-digestible dietary fiber, carbohydrates and in some cases protein (Minamoto *et al*. 2019). The most important SCFAs are propionate, butyrate, and acetate. These products have a positive effect on host health in multiple ways. They can modulate inflammation in the GI tract by decreasing some proinflammatory cytokines and increasing some anti-inflammatory cytokines. They can also activate a transcription factor called Foxp3 which suppresses and regulates inflammation. Furthermore, SCFAs provide an acidic luminal environment which can prevent the overgrowth of pH-sensitive pathogenic bacteria. In addition to that, butyrate is the preferred energy source of enterocytes.

### 2.1.2 The effect of diet on the gut microbiota

In their natural state, the diet of dogs mainly consists of meat (Pilla & Suchodolski 2020). Traditional extruded dry dog food contains both animal and vegetable products and generally contains a high load of carbohydrates in comparison to meat.

The macronutrients of the ingredients seem to be more important than their kingdom of origin. In a study by Bresciani *et al* (2018), they gave dogs Animal protein-free diets (APFD), but with similar macronutrient composition as traditional extruded diets, and found no changes in the faecal microbiota of healthy dogs.

Raw meat food diets significantly differ in macronutrient content compared with traditional extruded diets, including more protein and less fiber and carbohydrates (Pilla & Suchodolski 2020). Studies have seen that dogs fed with raw diets had a decrease in the total number of bacteria in the phylum Firmicutes and Bacteroidetes, most of the affected genera produce SCFAs and digest dietary fiber. Furthermore, bacteria from the phylum Proteobacteria Fusobacteria as well as two genera from the phylum Firmicutes (*Laktinobacillus* and *Clostridium*) were increased.

Butyrate kinase (*buk*) genes have been linked to *Clostridium perfringens* and *Clostridium dificille* in dogs eating a carnivorous diet, suggesting that they can produce butyrate from protein sources which is vital in a carnivorous diet (Vital *et al*. 2015).

## 2.2 The gut microbiota and GI disease

Several studies have seen a correlation between the presence or absence of specific bacteria as well as the dysbiosis index and GI disorders (Suchodolski *et al*. 2012, Vázquez-Baeza *et al*. 2016, Xenoulis *et al.* 2008, Suchodolski *et al.* 2010).

In a study by Minamoto *et al*. (2019), they found that dogs with chronic enteropathy (CE), relative to healthy controls (HC), had a higher dysbiosis index, decreased microbial diversity, and a decreased amount of Bacteroidetes, *Blautia* spp., *Faecalibacterium* spp., *Fusobacterium* spp., *Turicibacter* spp., and *C. hiranonis.* All these bacteria except for *Turicibacter* spp. had a positive correlation with the concentration of the SCFA propionate. They also found that dogs with CE had lower amounts of SCFAs and higher amounts of *Bifidobacterium* spp., *Lacto-bacillus* spp., *Streptococcus* spp., and *E. coli* compared with HC. Most of these bacteria had a negative correlation with the concentration of propionate.

A study using meta-analyses to identify biomarkers for GI functionality in dogs systematically reviewed 27 randomized controlled and case-controlled trials, where 815 healthy dogs and 786 with GI disease were included (Félix *et al*. 2022). They found a significant decrease in the abundance of *Faecalibacterium, Turicibacter, C. hiranonis, Blautia,* and *Fusobacterium* as well as a significant increase in the abundance of *E. coli* in dogs with GI disease compared to HC. Dogs with GI disease also had a lower α-diversity and concentrations of fecal propionate and secondary bile acids, a greater dysbiosis index and concentrations of fecal calprotectin and primary bile acids.

*Faecalibacterium prausnitzii, Blautia, Turicibacter,* and *Fusobacterium* are important in the gut microbiome partly due to their ability to produce SCFAs (Pilla & Suchodolski 2020, Ziese & Suchodolski 2021). *C. hiranonis* transform primary bile acids into secondary bile acids in the colon (Ziese & Suchodolski 2021). Secondary bile acids have anti-inflammatory properties, it also inhibits the growth of *C. difficile, C. perfringens,* and *E. coli.*

### 2.2.1 Chronic enteropathy (CE)

In general, the diagnosis CE is given if the patient has had clinical signs of GI disease for more than 3 weeks and the cause is unknown (Idiopathic) (Dandrieux 2016). It can be subdivided into 4 groups based on response to treatment; Food responsive enteropathy (FRE), Antibiotic responsive enteropathy (ARE), Immunosuppressant-responsive enteropathy (IRE), and Non-responsive enteropathy (NRE). FRE is the most common group. They are clinically very similar, and no effective biomarkers have been found to differentiate between them to date (Alshawaqfeh *et al*. 2017).

### 2.2.2 Acute diarrhea (AD)

Dogs with both acute non-haemorrhagic diarrhea (NHD) and acute haemorrhagic diarrhea syndrome (AHDS) have dysbiosis (Suchodolski *et al.* 2012). They have a decrease in bacteria like *Blautila* spp., *Ruminococcacaea, Faecalibacterium* spp. and, *Turicibacter* spp. Dogs with AHD also have an increase in the number of bacteria in the genus *Suterella and Clostridium* and the phylum Fusobacteria.

*C. perfringens* is a commensal and may even be beneficial for a carnivore due to its ability to produce butyrate from protein (Vital *et al*. 2015). However, *C. perfringens* type A isolates with the toxin-producing genes *netE* and *netF* are significantly associated with AHDS (Sindern *et al*. 2019).

### 2.2.3 Dysbiosis

Dysbiosis is a disease-inducing imbalance of the gut microbiota that can be caused by a reduction in microbial diversity, expansion of new bacterial groups, and large shifts in the ratio between bacterial phyla (Weiss & Hennet 2017). Studies have seen that dysbiosis is present in both acute enteropathy (Suchodolski *et al*. 2012, Heilmann *et al*. 2017) and chronic enteropathy (Xenoulis *et al*. 2008, Suchodolski *et al*. 2012, Vázquez-Baeza *et al*. 2016, Alshawaqfeh *et al*. 2017, Minamoto *et al*. 2019). The abundance percentages of each taxon differ between studies in this field, most taxa are however consistently increased or decreased in specific disease phenotypes.

The availability of oxygen in the intestinal lumen might be responsible for some microbial changes observed in dysbiosis (Pilla & Suchodolski 2020). Free oxygen in the lumen can increase during inflammation. This negatively affects strict anaerobes and drives the expansion of facultative anaerobes, mainly members of the *Enterobacteriaceae* family, which is a common marker of dysbiosis.

A study by Alshawaqfeh *et al*. (2017) developed a dysbiosis index (DI) to aid in differentiating between healthy dogs and dogs with CE. They used several mathematical models and PCR assays to find the panel with the highest discriminatory power. Their final qPCR panel consisted of eight bacterial groups that are commonly affected in dogs with CE (total bacteria, *Faecalibacterium*, *Turicibacter*, *Escherichia coli*, *Streptococcus*, *Blautia*, *Fusobacterium*, and *Clostridium hiranonis*). They achieved a sensitivity of 74% and a specificity of 95%. A DI below 0 indicates normobiosis, a positive value indicates dysbiosis, and a higher positive value indicates more dysbiosis than a lower positive number. This means that the DI may be used to monitor disease progression and response to treatment.

### 2.2.4 Calprotectin

Calprotectin is a protein complex belonging to the S100/calgranulin family (Grellet *et al*. 2013). It is present mainly in neutrophils, but also in monocytes and reactive macrophages. It is used as a biomarker in human medicine to limit the need for invasive diagnostics and to aid in evaluating the progression of intestinal inflammation and response to treatment. It is a $Ca^{2+}$ binding protein and has been associated with acute and chronic inflammation. When it is released extracellularly, it functions as an endogenous danger-signalling molecule (Heilmann *et al.* 2018) and triggers inflammation by binding to TLRs (Foell *et al.* 2007).

Fecal calprotectin is increased in dogs with chronic diarrhea, especially in dogs with histological intestinal lesions in relation to HC (Grellet *et al.* 2013). It is also

correlated with the severity of those lesions. It can aid in differentiating between dogs with IRE and FRE or ARE, especially in the combination with CRP and CCECAI scores (Heilmann *et al.* 2018). A study investigating dogs with AHDS found that they also had increased levels of fecal calprotectin (Heilmann *et al.* 2017). Within 3 days of treatment, the levels had significantly decreased.

# 3. Material and Methods

## 3.1 Study population and sampling

Stool samples were collected from healthy dogs arriving at SLU Uppsala to donate blood, and from dogs with GI disorders at the University Animal Hospital (UDS). Samples collected in the afternoon were kept in a fridge at 8°C for less than 24 hours. The feaces were marked and a pea-sized sample was put into a tube for microbial analyses. The stool was kept at -20°C until the analysis of calprotectin. The tubes were kept for 1-3 days at -20°C and then moved to -80°C until the microbial analyses. The samples were sent to the National Veterinary Institute (SVA) for bacterial culture and analysis of calprotectin. Blood samples were taken in conjunction with their visit. The owners answered a survey containing questions about their dog's health related to GI disorders. The same survey will be used in a sister study where stool samples will be collected from dogs before, and 3 weeks after the start of cytostatic treatment.

## 3.2 Analysis of stool

### 3.2.1 Analyses of microbiota

The following method will be used for the analyses of the microbiota at a later stage of this study:

**Faeces sample preparation**

*DNA isolation*

DNA isolation from canine faeces was performed as described in (Söder *et al*. 2022). In summary; Total DNA was isolated from 0.2 g of faeces using the QIAamp DNA Mini Kit (QIAGEN, GmbH, Hilden, Germany), according to manufacturer's instruction, but for lysis of bacterial cells, 0.1 mm zirconium/silica beads (Biospec Products INC, Bartlesville, OK, USA) was used instead of enzymatic lysis.

Again, the same methodology was used as earlier described in (Söder *et al*. 2022). Very briefly, 16S rRNA gene amplicons were generated and sequenced by Illumina

sequencing. Polymerase chain reaction (PCR; using Phusion® High-Fidelity PCR chemistry (New England Biolabs, Ipswich, MA, USA)) amplicons were generated with standard commercially available primers (515F and 806R, amplifying part of the 16S gene). Purification with Qiagen Gel extraction kit (Qiagen) followed and then quantification into equimolar amounts.

The amplicon library was processed and sequenced on an Illumina HiSeq platform 2500 at Novogene (Beijing, China). Paired-end sequence reads were merged using FLASH (Version 1.2.7) UPARSE software (Version 7.0.1001) was used to cluster the remaining sequences into operational taxonomic units (OTUs), using ≥ 97% homology as the threshold for classification as an OTU. For annotation, the SSU rRNA database SILVA (Quast *et al.* 2013) was used (licensed under Creative Commons Attribution 4.0 (CC-BY 4.0).

### 3.2.2 Analyses of calprotectin

At a later stage of this study, calprotectin will be analysed using a dog calprotectin ELISA kit from Abbexa. The antibodies in this kit are dog-specific and polyclonal (Abbexa). It is based on sandwich enzyme-linked immuno-sorbent assay technology. It recognizes the target at the Glu25-Glu136 amino acid sequence. For step-by-step instructions, read the assay procedure on Abbexa's product page.

## 3.3 Literature search

The studies were collected from Primo, PubMed and Science Direct. The search terms *Dogs*, *Dog* or *Canine* were combined in different ways with *gastrointestinal disease/disorders*, *healthy*, *calprotectin*, *gut microbiota*, *microbiome*, *SCFAs*, *dysbiosis*, *chronic*, *acute*, *hemorrhagic*, *diarrhea*, *CE*, *diet* and *feces*. The list of references was also examined in relevant articles.

A few peer-reviewed review articles were also included due to the difficulty of getting access to or finding the source material.

# 4. Results

So far, faeces from 22 dogs have been sampled. As previously mentioned, the analyses of both the gut microbiota and calprotectin will be performed at a later stage.

## 4.1 Survey

Of the 22 dogs sampled from, 12 were healthy controls, 2 suffered from acute gastro enteritis and 8 suffered from chronic gastrointestinal disorders.

100% of the dogs with GI disorders and 8% of HC had been treated at least once during the last year due to vomiting and or diarrhoea. The most common treatments were dietary modulation (72%) and pre-and/or probiotics (64%).

50% of the owners of dogs with GI disorders and 8% of the owners of HC perceived their dog to have a mildly affected level of activity. 40% of the owners of dogs with GI disorders perceived their dog to have a mildly decreased quality of life in comparison to 0% of HC.

90% of dogs with GI disorders and 67% of HC were fed traditional dry and/or wet dog food as their main diet. 10% of dogs with GI disorders and 8% of HC were fed exclusively raw meat food as their main diet. 0% of dogs with GI disorders were fed raw meat food in combination with extruded diets as their main diet in comparison to 25% of HC. 30% of dogs with GI disorders and 8% of HC were given dietary supplements daily.

To view the complete result of the survey, see appendix 1. To view the survey, see appendix 2.

# 5. Discussion

The original purpose of this study was to collect fecal samples for the analysis of the intestinal microbiota and calprotectin. However, due to practical constraints, further sampling and analysis will be performed at a later stage. Therefore, this paper cannot as of yet provide a comprehensive review of these factors in relation to host health. Consequently, this study will focus on the potential of the gut microbiota and calprotectin in gastrointestinal disorders.

## 5.1 The diagnostic value of the gut microbiota

Analysis of fecal biomarkers for diagnostic reasons is particularly useful because stool sampling is non-invasive, and therefore less stress inducing for the patient than most traditional diagnostic tools.

The dysbiosis index is an example of a way to utilize the gut microbiota as a diagnostic tool. As previously mentioned, the qPCR panel consists of total bacteria, *Faecalibacterium*, *Turicibacter*, *Escherichia coli*, *Streptococcus*, *Blautia*, *Fusobacterium*, and *Clostridium hiranonis* which all have been shown to be affected in CE (Alshawaqfeh *et al.* 2017). A meta-analysis study by Félix *et al*. (2022) observed a significant difference in the dysbiosis index between HC and dogs with GI disorders where the HC had a lower DI. However, they did not observe a significant difference between dogs with GI disorders before and after treatment. They did albeit only have two studies in their meta-analysis that investigated the DI before and after treatment, with a total of 38 dogs, eight of which had acute clinical signs. One interesting approach for a future study would be to evaluate how effective the DI is at differentiating between HC and dogs with acute disorders since it was specifically developed for dogs with CE (Alshawaqfeh *et al*. 2017).

In addition to the dysbiosis index, identifying and measuring certain bacteria sensitive to gut homeostasis changes can be a valuable diagnostic for monitoring gastrointestinal functionality. The previously mentioned meta-analysis study found that *Blautia* spp., *Turicibacter* spp. and *Faecalibacterium* spp. had the highest discriminatory power to differentiate between HC and dogs with GI disease, independent of disease phenotype (Félix *et al*. 2022).

## 5.2 Therapeutic approaches to dysbiosis

Since the microbiome plays an important role in maintaining a healthy gastro-intestinal tract, the normalisation of the microbiota is an important therapeutic target (Ziese & Suchodolski 2021). Dysbiosis can cause a disruption of the intestinal barrier, increasing the risk of the translocation of pathogens. In addition to that, it can also promote pro-inflammatory processes.

The therapeutic approaches available today are dietary modulation, pre-and probiotics, antibiotics, and fecal matter transplant (FMT) (Ziese & Suchodolski 2021). However, the underlying disease must of course be taken into account and treated aswell. For example, in cases of chronic enteropathy, the inflammation must be treated for improvement of the disease process. Hopefully, after normobiosis is achieved, the products of the beneficial bacteria and their anti-inflammatory and immunomodulatory properties will keep the gastrointestinal system in homeostasis.

## 5.3 The diagnostic value of calprotectin

In a study by Heilmann *et al*. (2018), they found that calprotectin levels are higher in the feces of dogs with IRE than in dogs with FRE and ARE, but significance was not reached, probably due to the low number of patients. However, in combination with serum CRP concentration and CCECAI scores, the ability to differentiate between these conditions greatly increased.

In a study by Grellet *et al*. (2013), they found that a cut of value of 48.9 μg/g had a moderate sensitivity (53.3%) and a high specificity (91.7%) to predict the risk of clinical relapse. They also found that the levels of fecal calprotectin were significantly higher in dogs with both chronic diarrhea and histological lesions than in dogs who only had chronic diarrhea.

Five studies evaluating fecal calprotectin were included in the meta-analyses of Félix *et al*. (2022). They found that the levels of calprotectin were significantly higher in dogs with gastrointestinal disease compared to healthy controls.

Considering the fact that Calprotectin is a protein found mainly in neutrophils, further studies are needed to investigate the levels of fecal calprotectin in relation to inflammatory infiltrates.

## 5.4 Problems and limitations

### 5.4.1 The survey

As for all surveys, the survey of this study is associated with problems and limitations. For starters, the questions of the survey can be misinterpreted by the owners. They also risk recall bias when answering questions about previous events. Furthermore, they might not be aware of the medical definition of the conditions asked about in the survey, they might, for example, think their dog vomited when it in fact expectorated. In addition to that, the so-called ‘’clever Hans’’ effect may also be in effect, where the owners answer as they think we want them to answer. Therefore, the answers must be interpreted with caution.

### 5.4.2 Population size

Because this is a pilot study, the population size will be relatively small. The aim is to sample 40 dogs, 20 healthy and 20 with GI disorders to evaluate if the method is logistically possible and if the ELISA used to analyze calprotectin is reliable. A larger population size would of course be preferable. The trends seen in this study can however elucidate interesting focuses for future studies on this topic.

### 5.4.3 Sampling

One drawback with letting the owners collect the stool is the risk of accidentally getting earthbound bacteria in the sample. That risk of course increases when the consistency of the stool decreases.

Another drawback is that feaces were kept at room temperature for different amounts of time depending on if it was collected at home or at the clinic. If they brought samples from home, the travel time from home to the clinic also varied. It will however be interesting to see if this will noticeably affect the results of the analyses within the different groups.

# References


Agria (2019). *5 vanligaste diagnoserna hos hunden.* Agria Djurförsäkring. http://www.agria.se/hund/artiklar/sjukdomar-och-skador/5-vanligaste-diagnoserna-hos-hunden/ [2022-11-08]

Abbexa (2022). *Dog Calprotectin ELISA kit.* https://www.abbexa.com/calprotectin-calpro-elisa-kit [2022-10-24]

Alshawaqfeh, M., Wajid, B., Minamoto, Y., Markel, M., Lidbury, J., Steiner, J., Serpedin, E. & Suchodolski, J. (2017) A dysbiosis index to assess microbial changes in fecal samples of dogs with chronic inflammatory enteropathy. *FEMS Microbiology Ecology*, 93(11). DOI:10.1093/femsec/fix136.

AVMA (2006). *Top 10 reasons pets visit veterinarians*. American Veterinary Medical Association. https://www.avma.org/javma-news/2006-09-01/top-10-reasons-pets-visit-veterinarians [2022-09-14]

Bresciani, F., Minamoto, Y., Suchodolski, J.S., Galiazzo, G., Vecchiato, C.G., Pinna, C., Biagi, G. & Pietra, M. (2018) Effect of an extruded animal protein-free diet on fecal microbiota of dogs with food-responsive enteropathy. *Journal of Veterinary Internal Medicine*, 32(6), 1903–1910. DOI:10.1111/jvim.15227.

Dandrieux, J.R.S. (2016). Inflammatory bowel disease versus chronic enteropathy in dogs: are they one and the same? *Journal of Small Animal Practice*, 57, 589–599. DOI: 10.1111/jsap.12588

Foell, D., Wittkowski, H., Vogl, T. & Roth, J. (2007) S100 proteins expressed in phagocytes: a novel group of damage-associated molecular pattern molecules, *Journal of Leukocyte Biology*, 81 (1). 28–37. DOI:10.1189/jlb.0306170.

Félix, A.P., Souza, C.M.M. & de Oliveira, S.G. (2022). Biomarkers of gastrointestinal functionality in dogs: A systematic review and meta-analysis. *Animal Feed Science and Technology*, 283, 115183. https://doi.org/10.1016/j.anifeedsci.2021.115183

Grellet, A., Heilmann, R. M., Lecoindre, P., Feugier, A., Day, M. J. & Peeters, D. (2013) Fecal calprotectin concentrations in adult dogs with chronic diarrhea, *American Journal of Veterinary Research*, 74 (5). 706–711. DOI:10.2460/ajvr.74.5.706.

Heilmann, R. M., Berghoff, N., Mansell, J., Grützner, N., Parnell, N. K., Gurtner, C., Suchodolski, J. S. & Steiner, J. M. (2018) Association of fecal calprotectin concentrations with disease severity, response to treatment, and other biomarkers in dogs with chronic inflammatory enteropathies, *Journal of Veterinary Internal Medicine*, 32 (2). 679–692. DOI:10.1111/jvim.15065.

Heilmann, R. M., Guard, M. M., Steiner, J. M., Suchodolski, J. S. & Unterer, S. (2017) Fecal markers of inflammation, protein loss, and microbial changes in dogs with the acute hemorrhagic diarrhea syndrome (AHDS), *Journal of Veterinary Emergency and Critical Care*, 27 (5). 586–589. DOI:10.1111/vec.12636.

Minamoto, Y., Minamoto, T., Isaiah, A., Sattasathuchana, P., Buono, A. & Rangachari, V. R. (2019) Fecal short-chain fatty acid concentrations and dysbiosis in dogs with chronic enteropathy, *Journal of Veterinary Internal Medicine*, 33 (4). 1608–1618. DOI:10.1111/jvim.15520.

Pilla, R. & Suchodolski, J.S. (2020). The role of the canine gut microbiome and metabolome in health and gastrointestinal disease. *Frontiers in Veterinary Science*, 6, 498. https://doi.org/10.3389/fvets.2019.00498

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. & Glöckner, F.O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41 (D1): D590-D596. https://www.arb-silva.de/ [2023-03-07]

Sindern, N., Suchodolski, J.S., Leutenegger, C.M., Mehdizadeh Gohari, I., Prescott, J.F., Proksch, A.-L., Mueller, R.S., Busch, K. & Unterer, S. (2019) Prevalence of Clostridium perfringens netE and netF toxin genes in the feces of dogs with acute hemorrhagic diarrhea syndrome, *Journal of Veterinary Internal Medicine*, 33 (1). 100–105. DOI:10.1111/jvim.15361.

Suchodolski, J.S., Xenoulis, P.G., Paddock, C.G., Steiner, J.M. & Jergens, A.E. (2010). Molecular analysis of the bacterial microbiota in duodenal biopsies from dogs with idiopathic inflammatory bowel disease. *Veterinary Microbiology*, 142 (3), 394–400. https://doi.org/10.1016/j.vetmic.2009.11.002

Suchodolski, J.S. (2011a). COMPANION ANIMALS SYMPOSIUM: Microbes and gastrointestinal health of dogs and cats1, *Journal of Animal Science*, 89 (5). 1520–1530. DOI:10.2527/jas.2010-3377.

Suchodolski, J.S. (2011b). Intestinal microbiota of dogs and cats: a bigger world than we thought. *The Veterinary Clinics of North America. Small Animal Practice*, 41 (2), 261–272. https://doi.org/10.1016/j.cvsm.2010.12.006

Suchodolski, J.S., Markel, M.E., Garcia-Mazcorro, J.F., Unterer, S., Heilmann, R.M. & Dowd, S.E. (2012). The fecal microbiome in dogs with acute diarrhea and idiopathic inflammatory bowel disease, *PLOS ONE*, 7 (12) e51907. DOI:10.1371/journal.pone.0051907.

Söder, J., Wernersson, S., Höglund, K., Hagman, R., Lindåse, S. & Dicksved, J. (2022). Composition and short-term stability of gut microbiota in lean and spontaneously overweight healthy Labrador retriever dogs. *Acta Veterinaria Scandinavica*, 64 (1), 8. https://doi.org/10.1186/s13028-022-00628-z

Vital, M., Gao, J., Rizzo, M., Harrison, T. & Tiedje, J.M. (2015). Diet is a major factor governing the fecal butyrate-producing community structure across Mammalia, Aves and Reptilia, *The ISME Journal*, 9 (4). 832–843. DOI:10.1038/ismej.2014.179.

Vázquez-Baeza, Y., Hyde, E.R., Suchodolski, J. S. & Knight, R. (2016) Dog and human inflammatory bowel disease rely on overlapping yet distinct dysbiosis networks, *Nature Microbiology*, 1 (12). 16177. DOI:10.1038/nmicrobiol.2016.177.

Weiss, G. A. & Hennet, T. (2017) Mechanisms and consequences of intestinal dysbiosis, *Cellular and Molecular Life Sciences*, 74 (16). 2959–2977. DOI:10.1007/s00018-017-2509-x.

Willard, M.D. (2019). Digestive system disorders. In: Nelson, R. W., & Couto, C. G. (eds.). *Small Animal Internal Medicine.* 6th ed. Philadelphia: Elsevier - Health Sciences Division, pp 400.

Xenoulis, P.G., Palculict, B., Allenspach, K., Steiner, J.M., Van House, A.M. & Suchodolski, J.S. (2008). Molecular-phylogenetic characterization of microbial communities imbalances in the small intestine of dogs with inflammatory bowel disease. *FEMS Microbiology Ecology*, 66 (3), 579–589. https://doi.org/10.1111/j.1574-6941.2008.00556.x

Ziese, A.-L. & Suchodolski, J.S. (2021). Impact of changes in gastrointestinal microbiota in canine and feline digestive diseases. *Veterinary Clinics of North America: Small Animal Practice*, 51 (1), 155–169. https://doi.org/10.1016/j.cvsm.2020.09.004

# Popular Science Summary

There are many different reasons for gastrointestinal disease in our dogs. These diseases can manifest themselves in a lot of different ways, for example, diarrhea, vomiting, nausea, and lack of appetite. It can be challenging for the veterinarian to find the cause of the problem and treat it swiftly using the diagnostics available today. Some of the diagnostics used can also be stressful for the dog.

The gut microbiome consists of an extreme number of microorganisms, they might even outnumber our own cells. They of course interact with the cells in our intestines and greatly affect our health. The beneficial bacteria in our gut help us digest our food, and their biproducts can for example be used for energy by our intestinal cells, decrease the risk of inflammation, and inhibit the growth of dangerous bacteria.

If this ecosystem in our intestines is put out of balance, for example by diet, antibiotics, auto immune disease, or toxins, dysbiosis can occur which can lead to previously mentioned clinical signs. However, analyzing the feces of our dogs can help us understand what changes occur in the microbiota during dysbiosis. If we can find what bacteria are affected and if certain inflammatory markers are increased, we could potentially give faster diagnostics, better prognostics, and treatment.

In this study, we collected fecal samples from both healthy dogs and dogs with gastrointestinal disorders to investigate any correlations between the changes in the microbiota, inflammatory markers, and what disorders the dogs suffered from.

We also asked the owners to answer a survey about their dogs' eating habits, health status, symptoms of recent gastrointestinal disease, medical treatment, and perceived quality of life to investigate any correlations between these factors and their microbiome.

However, due to unforeseen events, the analysis of the feces will not be performed until a later stage of this study.

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| | Patient | A | B | C | D | E | F | G | H | I | J | K |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Datum för insamling | | 2022-09-22 | 2022-09-22 | 2022-09-22 | 2022-10-06 | 2022-10-06 | 2022-10-19 | 2022-10-19 | 2022-10-20 | 2022-10-20 | 2022-11-01 | 2022-11-02 |
| Allmän info | Ras | Flat coated retriver | Flat coated retriver | Labrador retriever | Labrador retriever | Labrador retriever | Blandras | Malinois/Belgisk vallhund | Dalmatin | Flat coated retriver | Schäfer | Långhårig collie |
| | Född (yyyy-mm-dd) | 2017-02-24 | 2019-09-10 | 2016-09-05 | 2017-01-01 | 2018-12-18 | 2013-11-01 | 2019-04-22 | 2018-03-18 | 2017-03-16 | 2018-01-01 | 2021-01-18 |
| | Kön | Hona | Hane | Hane | Hane | Hane | Hona | Hona | Hona | Hane | Hane | Hane |
| | Vikt (kg) | 27,7 | 36 | 31,4 | 32,7 | 40,3 | 20,6 | 25,9 | 27,1 | 42 | 32 | 20,7 |
| | Tillstånd | Blodgivare | Blodgivare | Blodgivare | Blodgivare | Blodgivare | Akut GE | Akut hemmorhagisk diarré | Blodgivare | Blodgivare | Blodgivare | Kroniska GI problem |
| Matvanor | | | | | | | | | | | | |
| 1 | Huvudsaklig kost | Hills torrfoder | Brit Care Sensitive torrfoder | Magnussons torrfoder | Magnussons vuxen torrfoder | Granngårdens spannmålsfria torrfoder, rått färskt kött med och utan ben | R/C mobility | R/C Gastro intestinal torrfoder | Hills torrfoder | Hills light torrfoder | R/C german shepherd adult torrfoder | Furry friends torrfoder |
| 2 | Godis/tuggben/matrester | Godbitar | Tuggben, godbitar, frukt (äpple) | Tuggben, märgben, matrester | Godbitar, matrester | Märgben, leksaker fyllda med godis, torkat kött, grönsaker, frukter | Tuggbitar för munnhälsa, matrester | Ej svarat | Tuggben, hundgodis, köttbitar | Morötter | Kong med fryst färskfoder (nordic) | Tuggben, hundgodis, matrester |
| 3 | Fodertillskott | Nej | Nej | Nej | Boswellia Serrata 1gång/dag | Nej | Nej | Ej svarat | Nej | Nej | Nej | Kokosolja 1gång/d, probiotika ibland |
| Kräkning | | | | | | | | | | | | |
| 1 | Senaste året | Ja | Nej | Ja | Nej | Ja | Ja (samma vecka) | Ja | Nej | Ja | Nej | Ja |
| 2 | Flera episoder vid olika tillfällen | Nej | N/A | Nej | N/A | Ja | Nej | Ej svarat | N/A | Ja | N/A | Ja |
| 3 | Duration (dagar) | 1 | N/A | 1 | N/A | 1 | 3+ | Ej svarat | N/A | 1 | N/A | 3+ |
| 4 | Behandling senaste året | Nej | N/A | Nej | N/A | Nej | Ja | Ej svarat | N/A | Nej | N/A | Ja |
| 5 | Typ av behandling | N/A | N/A | N/A | N/A | N/A | Foderbyte, Pro-kolin | Ej svarat | N/A | N/A | N/A | Foderbyte, medicinering (probiotika, maropitant) |
| Diarré | | | | | | | | | | | | |
| 1 | Senaste året | Ja | Nej | Nej | Nej | Ja | Ja (samma vecka) | Ja | Nej | Ja | Ja | Ja |
| 2 | Flera episoder vid olika tillfällen | Ja | N/A | N/A | N/A | Ja | Nej | Ej svarat | N/A | Ja | Ja | Ja |
| 3 | Duration (dagar) | 2 | N/A | N/A | N/A | 2 | 3+ | 2 | N/A | 1 | 1 till 2 | 3+ |
| 4 | Behandling senaste året | Ja | N/A | N/A | N/A | Nej | Ja | Ja | N/A | Nej | Nej | Ja |
| 5 | Typ av behandling | Vila, foderbyte, probiotika | N/A | N/A | N/A | N/A | Foderbyte, Pro-kolin | Pro-kolin, skonkost | N/A | N/A | N/A | Foderbyte, medicinering (probiotika, maropitant) |
| Sjukdom & medicinering | | | | | | | | | | | | |
| 1 | Underliggande sjukdom | Nej | Nej | Nej | Nej | Ja | Nej | Nej | Nej | Nej | Nej | Nej |
| 2 | Typ av sjukdom | N/A | N/A | N/A | N/A | Otit | N/A | N/A | N/A | N/A | N/A | N/A |
| 3 | Duration av sjukdom | N/A | N/A | N/A | N/A | Ej svarat | N/A | N/A | N/A | N/A | N/A | N/A |
| 4 | Behandling mot sjukdom | N/A | N/A | N/A | N/A | Ej svarat | N/A | N/A | N/A | N/A | N/A | N/A |
| 5 | Vilken behandling | N/A | N/A | N/A | N/A | Ej svarat | N/A | N/A | N/A | N/A | N/A | N/A |
| 6 | Antibiotika | Nej | Nej | Nej | Nej | Nej | Nej | Ej svarat | Nej | Nej | Nej | Ja |
| 7 | Andra mediciner i hemmet | Nej | Nej | Nej | Nej | Nej | Nej | Nej | Nej | Nej | Nej | Ja |
| 8 | Typ av mediciner i hemmet | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | Ursofalk |
| Övriga frågor | | | | | | | | | | | | |
| | Aktivitetsnivå (1: normal, 2: lindrigt trött, 3: måttligt trött, 4: mycket trött) | 1 | 1 | 1 | 1 | 2 | 1 | Ej svarat | 1 | 1 | 1 | 2 |
| | Livskvalité (1: normal, 2: lindrigt nedsatt, 3: kraftigt nedsatt) | 1 | 1 | 1 | 1 | 1 | 1 | Ej svarat | 1 | 1 | 1 | 1 |

# Appendix 1

Spreadsheet summary of the complete results of the survey.

White: Healthy control, yellow: acute enteropathy, orange: chronic enteropathy

| | L | M | N | O | P | Q | R | S | T | U | V |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Datum för insamling | 2022-11-02 | 2022-11-03 | 2022-11-03 | 2022-11-03 | 2022-11-03 | 2022-11-07 | 2022-11-07 | 2022-11-08 | 2022-11-21 | 2022-11-21 | 2022-12-01 |
| Allmän info | Bearded collie | Golden retriever | Cane Corso | Grand danois | Storpudel | Mellanpudel | Howart | Schäfer | Dvärgpudel | Chodsky Pes | Boxer |
| | 2020-05-31 | 2020-10-16 | 2018-03-08 | 2020-05-01 | 2020-07-03 | 2018-11-11 | 2018-09-07 | 2017-08-01 | 2015-06-02 | 2014-05-24 | 2012-07-04 |
| | Hane | Hane | Hane | Hane | Hane | Hane | Hane | Hane | Hane | Hona | Hona |
| | 27,7 | 28,4 | 56,3 | 33,5 | 27,1 | 9 | 29,9 | 35,2 | 4,6 | 20,4 | 23 |
| | Kronisk enteropati | Blodgivare | Blodgivare | Blodgivare | Blodgivare | IBD | CE | PLE | Diarré | CE | PLE |
| Matvanor | | | | | | | | | | | |
| 1 | R/C sensitivity anka blöt, R/C GI lowfat | Rått färskt kött utan ben | Mush wild blötfoder, Hills vet essentials torrfoder, rått färskt kött med ben | Mush wild blötfoder, Hills vet essentials torrfoder, rått färskt kött med ben | Purina lamm torrfoder | Anallergenic + hypoallergenic torrfoder | Rått färskt kött utan ben + ris | Purina HA torrfoder | R/C GI blötfoder, R/C GI moderate calorie torrfoder | Specific CDD-HY torrfoder | R/C anallergenic blötfoder |
| 2 | Nej | Tuggben, tuggbitar för ökad munhälsa | Rökta oxben, kycklingfötter | Rökta oxben, kycklingfötter | Tuggben, hundgodis, matrester | Kong med blötlagt torrfoder | Hundgodis | Tuggben, tuggbitar för munhälsa | Nej | Nej | Nej |
| 3 | Nej | Nej | Nej | Nej | Nej | Nej | Psylliumfiber + morotspellets | Nej | Nej | Nej | Vivomixx 1 påse/d |
| Kräkning | | | | | | | | | | | |
| 1 | Ja | Nej | Nej | Nej | Ja | Ja | Ja | Ja | Ja | Nej | Ja |
| 2 | Ja | N/A | N/A | N/A | Nej | Ja | Ja | Ja | Nej | N/A | Ja |
| 3 | 3+ | N/A | N/A | N/A | 1 | 2 | 3+ | 3+ | 1 | N/A | 2 |
| 4 | Ja | N/A | N/A | N/A | Nej | Ja | Ja | Ja | ja | N/A | Nej |
| 5 | Foderbyte, medicinering, inskrivning | N/A | N/A | N/A | N/A | Foderbyte, medicinering | Foderbyte, andapsin | Vila | Medicinering, inskrivning, avmaskning med axilur | N/A | N/A |
| Diarré | | | | | | | | | | | |
| 1 | Ja | Nej | Nej | Nej | Ja | Ja | Ja | Ja | Ja | Ja | Ja |
| 2 | Ja | N/A | N/A | N/A | Nej | Ja | Ja | Ja | Ja | Ja | Ja |
| 3 | 3+ | N/A | N/A | N/A | 1 | 3+ | 2 | 2 | 3+ | 2 | 3+ |
| 4 | Nej | N/A | N/A | N/A | Nej | Ja | Ja | Nej | Ja | Ja | Ja |
| 5 | N/A | N/A | N/A | N/A | N/A | Foderbyte, medicinering | Foderbyte, andapsin | N/A | Inskrivning, pro-kolin | Pro-biotika | Foderbyte (till hydrolyserat), Medisinering (prednisolon) |
| Sjukdom & medicinering | | | | | | | | | | | |
| 1 | Ja | Nej | Nej | Nej | Nej | Ja | Ja | Ja | Nej | Ja | Ja |
| 2 | Kronisk enteropati | N/A | N/A | N/A | N/A | IBD | Kvallsterallergi, tidigare cystinsten | PLE | N/A | Allergi | PLE (misstänkt IBD eller neoplasi) |
| 3 | Mer än 1 år | N/A | N/A | N/A | N/A | Mer än 1 år | Mer än 1 år | Mer än 1 år | N/A | Mer än 1 år | Mindre än 6 mån |
| 4 | Ja | N/A | N/A | N/A | N/A | Ja | Ja | Ja | N/A | Ja | Ja |
| 5 | Hydroliserat lowfat foder, andapsin | N/A | N/A | N/A | N/A | Hypo + anallergenic torrfoder, Medrol (4mg/d), Imurel (25 mg 5ggr/v), Andapsin (3ggr/d vid behov) | Foder anpassat för cystinsten | Purina HA, B12 tillskott | N/A | Specific CDD-HY | R/C anallergenic blötfoder, vivomixx, MCT-olja, prednisolon |
| 6 | Nej | Nej | Nej | Nej | Nej | Nej | Nej | Nej | Nej | Nej | Nej |
| 7 | Ja | Nej | Nej | Nej | Nej | Nej | Nej | Nej | Nej | Ja | Nej |
| 8 | Mirtazapin, Cerenia | N/A | N/A | N/A | N/A | N/A | Cobalaplex 2 kapslar/d | N/A | N/A | B12 + folsyra | N/A |
| Övriga frågor | | | | | | | | | | | |
| | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 2 |
| | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 2 | 2 |

# Appendix 2

The complete survey.

**Min hunds mående**

***Kräkning***

*Ringa in de alternativ som passar in för din hund:*

1. Har din hund haft kräkningar under det senaste året?
    a. Ja
    b. Nej

*Om du svarat "Ja" på ovanstående fråga (fråga 1), svara då även på följande frågor om kräkning. Ringa in de alternativ som passar in för din hund och/eller skriv i fritext på linjen.*

2. Har din hund haft flera episoder av kräkningar vid olika tillfällen under det senaste året?
    a. Ja
    b. Nej

3. Hur länge kräktes din hund vid den senaste episoden av kräkning (en eller flera tillfällen per dag)?
    a. 1 dag
    b. 2 dagar
    c. 3 dagar eller fler

4. Har din hund behandlats för kräkning under det senaste året?
    a. Ja
    b. Nej

5. Om du svarat ja på fråga 4: vilken/vilka typ(er) av behandling(ar) genomförs/genomfördes?
    a. Vila
    b. Foderbyte
    c. Medicinering
    d. Inskrivning på djursjukhus för behandling
    e. Operation
    f. Annat: _____

***Diarré***

Ringa in alla alternativ som passar in.

1. Har din hund haft diarré under det senaste året?
    a. Ja
    b. Nej

*Om du svarat "Ja" på ovanstående fråga (fråga 1), svara då även på följande frågor om diarré. Ringa in de alternativ som passar in för din hund och/eller skriv i fritext på linjen.*

2. Har din hund haft flera episoder av diarréer vid olika tillfällen under det senaste året?
    a. Ja
    b. Nej

3. Hur länge hade din hund diarré vid den senaste episoden (en eller flera tillfällen per dag)?
    a. 1 dag
    b. 2 dagar
    c. 3 dagar eller fler

4. Har din hund behandlats för diarré under det senaste året?
    a. Ja
    b. Nej

5. Om du svarat ja på fråga 4: Vilken/vilka typ(er) av behandling(ar) genomförs/genomfördes?
    a. Vila
    b. Foderbyte
    c. Medicinering
    d. Inskrivning på djursjukhus för behandling
    e. Operation
    f. Annat: _____

***Sjukdomar & medicinering***

Ringa in alla alternativ som passar in.

1. Har din hund någon underliggande sjukdom?
    a. Ja
    b. Nej

*Om du svarat "Ja" på ovanstående fråga (fråga 1), svara då även på följande frågor om din hunds sjukdomar och medicinering. Ringa in de alternativ som passar in för din hund och/eller skriv i fritext på linjen.*

2. Vilken/vilka sjukdom/-ar har din hund? *(exempelvis allergi, IBD, njur- eller leversjukdom, hormonell sjukdom, hudsjukdom, hjärtsjukdom):*

_____
_____
_____

3. Hur länge har din hund haft denna sjukdom/dessa sjukdomar?
    a) Mindre än 6 månader
    b) Mer än 6 månader, mindre än 1 år
    c) Mer än 1 år

4. Står din hund på någon behandling mot denna sjukdom/dessa sjukdomar?
    a) Ja
    b) Nej

5. Om du svarat ja på fråga 4, vilken behandling står din hund på?
    a) Specialfoder, isåfall vilket/vilka?:
        _____
        _____
    b) Tillskott, isåfall vilket/vilka?:
        _____
        _____

5. (fortsättning)
    c) Medicinering, isåfall vilket/vilka?

    _____
    _____
    _____
    _____

    d) Annat:

    _____
    _____

6. Har din hund behandlats med antibiotika under de senaste 3 månaderna?
    a. Ja
    b. Nej

7. Behandlas din hund med några andra mediciner i hemmet utöver de som nämnts ovan (fråga 5 & 6) under det senaste halvåret?
    a. Ja
    b. Nej

8. Om du svarat ja på fråga 7, vilken/vilka mediciner behandlas din hund med? *(Inkludera inte de du redan har nämnt i fråga 5)*

    _____
    _____
    _____

**Hur bedömer du din hunds aktivitetsnivå just nu?**

1. Normal
2. Lindrigt trött
3. Måttligt trött, orkar endast gå upp för avföring och urinering samt för att äta.
4. Mycket trött, måste skrivas in på djursjukhus för att äta och få hjälp med urinerings- och avföringsbeteende.

**Hur upplever du hundens livskvalitet just nu?** *(hundens livssituation och välmående)*

1. Normal livskvalitet
2. Lindrigt nedsatt livskvalitet
3. Kraftigt nedsatt livskvalitet

***Tack för ditt deltagande!***

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