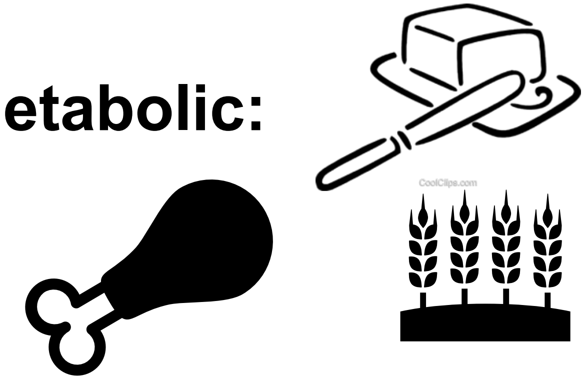
Join the Conversation: Comprehensive approach to microbiota analysis interpretation

Linda Toresson, DVM
Swedish Specialist in diseases of dogs & cats
Swedish Specialist in internal medicine
PhD

TEXAS A&M UNIVERSITY
Gastrointestinal
Laboratory

The intestinal microbiota executes many important functions

Metabolic:



Metabolites w. important intestinal and extraintestinal function (glucose homeostasis etc)

Primary Bile Acids

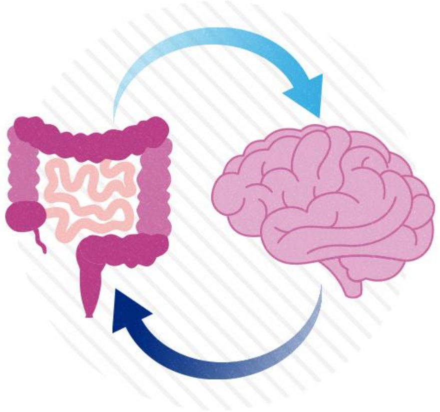
Secondary Bile Acids

Immune regulation

Mucosal defense

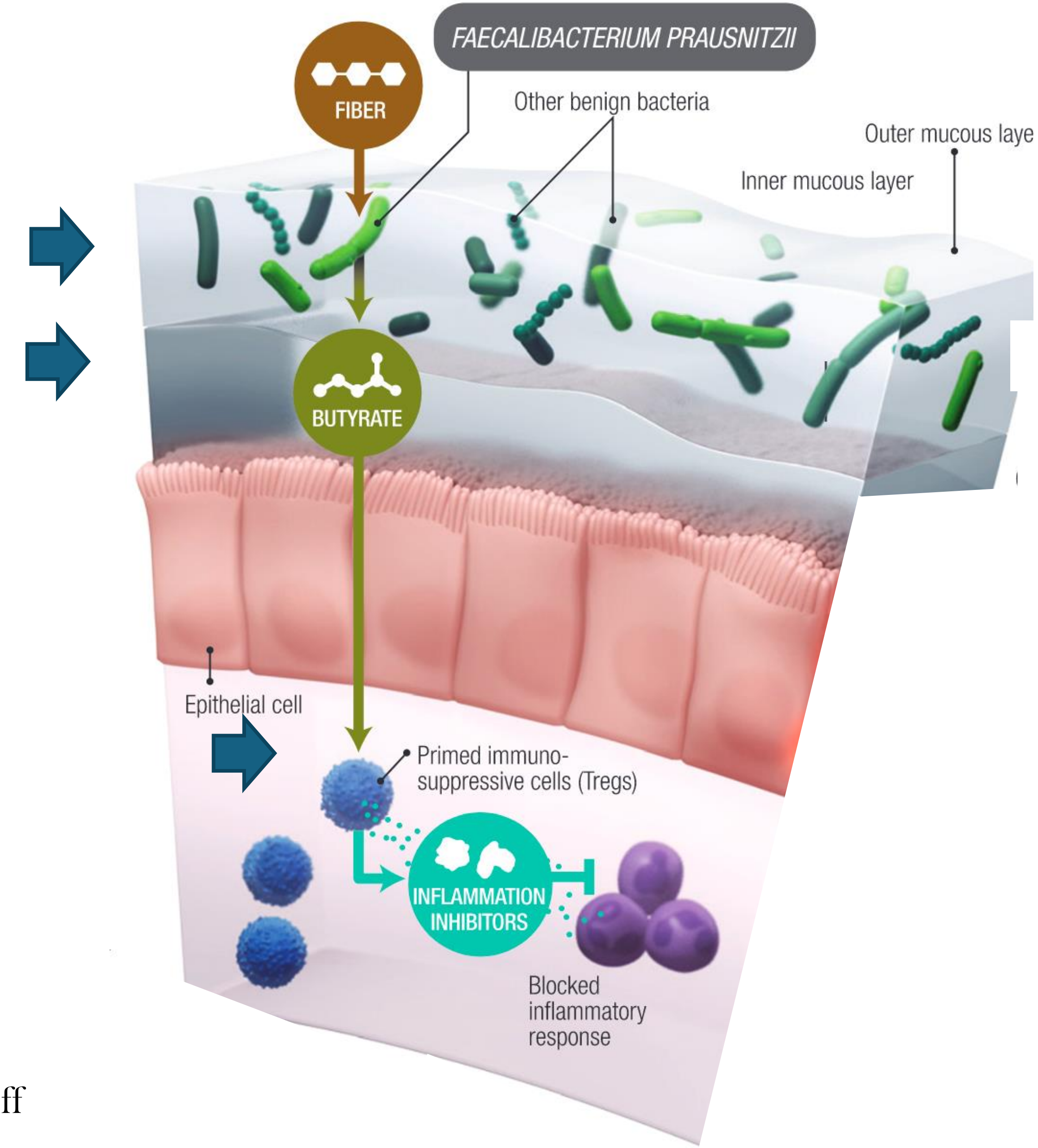
Integrity of mucosal barrier

Vitamin synthesis



Gut – Brain Axis

Etc…



M. Velasquez-Manoff

NATURE | VOL 518 | 26 FEBRUARY 2015

and obesity. Some of its relatives, called clostridial clusters, have similar properties

Dysbiosis in people with IBD

Alterations of resident microbial composition and function

Common features: decreased bacterial diversity, loss of core bacteria

- Significant reduction of beneficial microbes - ↓ short-chain fatty acid producing bacteria - *Faecalibacterium, Lachnospiraceae, Roseburia*, and *Ruminococcaceae*

- Increase of resident pathobionts, especially *Enterobacteraceae*

➢ Increased primary bile acids (BA) + decreased secondary BA and SCFAs (butyrate and propionate)

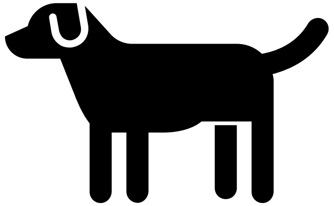
Beyond Random Fecal Microbial Transplants

Next Generation Personalized Approaches to Normalize Dysbiotic Microbiota for Treating IBD

R. Balfour Sartor, MD[a,b,*]

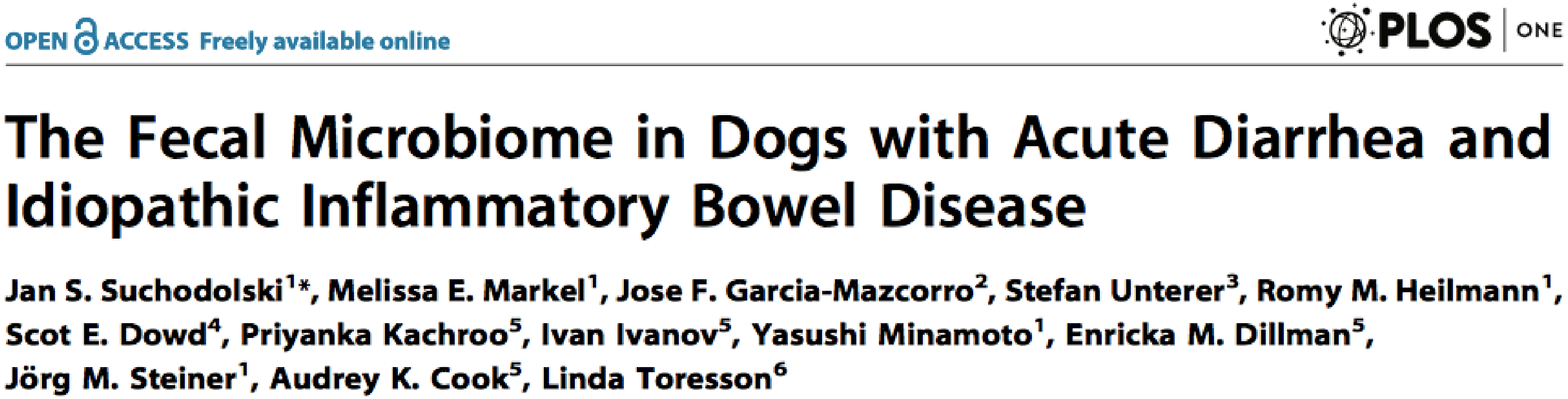
Gastroenerol Clin N Am 2024

Dysbios in CE dogs vs healthy:



- Less diverse microbiome – depletion, fewer bact. species
- Loss of important core bacteria required for proper microbiome function (*Faecalibacterium, P. hiranonis* etc)
- Significant reduction of beneficial microbes - ↓ short-chain fatty acid producing bacteria

(*Blautia* spp., *Faecalibacterium* spp., *Turicibacter* spp *Fusobacterium spp)*



OPEN ACCESS Freely available online

PLOS | ONE

The Fecal Microbiome in Dogs with Acute Diarrhea and Idiopathic Inflammatory Bowel Disease

Jan S. Suchodolski[1]*, Melissa E. Markel[1], Jose F. Garcia-Mazcorro[2], Stefan Unterer[3], Romy M. Heilmann[1], Scot E. Dowd[4], Priyanka Kachroo[5], Ivan Ivanov[5], Yasushi Minamoto[1], Enricka M. Dillman[5], Jörg M. Steiner[1], Audrey K. Cook[5], Linda Toresson[6]

Traditional bacterial culture

- Minor part of the intestinal microbiota culturable with standard culture techniques

- Consists mainly of anaerobes

Many bacterial species:
- have specific, complex nutritional needs

- rely on symbiotic relationships with other bacteria in the gut environment

"Jycke", mixed-breed dog, intact male, 7 Y/A

- Attacks of abdominal pain, lethargy, recurrent diarrhea for > 1 year
- Treated twice for hemolytic *E.coli* w. amoxicillin according to culture + sensitivity testing
- No improvement – dog owners went to a new vet

”Jycke”, mixed-breed dog, 7 Y/A

- New fecal sample:
- Neg. parasites
- 3rd course of amoxicillin...

Escherichia coli (haemolytic) 1)

Antibiogram org. no. 1)
Ampicillin S Amoxicillin S * Amoxicillin + Clavul S Cefalexin S Cefovecin S * Ceftiofur S Cefquinome S * Polymyxin B S Colistin S * Gentamicin S Tetracycline S stands for all tetracyclines Doxycycline S * Enrofloxacin S stands for all fluochino- lones Marbofloxacin S Pradofloxacin S * Nitrofurantoin S Sulfamethox./Trim. S also valid for other sulfonamide/trimeth.-combinations Chloramphenicol I stands for all phenicoles (e.g. Florphenicole) Imipenem S Strictly verify indication Meropenem S */ Strictly verify indication

"Jycke", mixed-breed dog

Next step: endoscopy

Histopathology report

- Mild diffuse lymphocytic-plasmacytic gastritis, presence of mild-moderate *Helicobact* spp.
- Mod.-severe LP chronic enteritis

➢ **Amoxicillin, metronidazole, PPi**

➢ **Referral**

”Jycke”, mixed-breed dog (currently 15 Y/A)

- **Treated with:**
- Hydrolyzed soy-based diet
- Probiotics
- Prebiotics

- **Immunosuppression – Prednisolone**

- **Addition 2nd line immunosuppressive:**
- **Azathioprine** ❌
- **Cyclosporine** ❌
- **Mycophenolate** ✅

4 courses of unnecessary antibiotics....

Received: 14 September 2020 | Accepted: 20 November 2020
DOI: 10.1111/jvim.15982

STANDARD ARTICLE

Journal of Veterinary Internal Medicine
Open Access
ACVIM
American College of Veterinary Internal Medicine

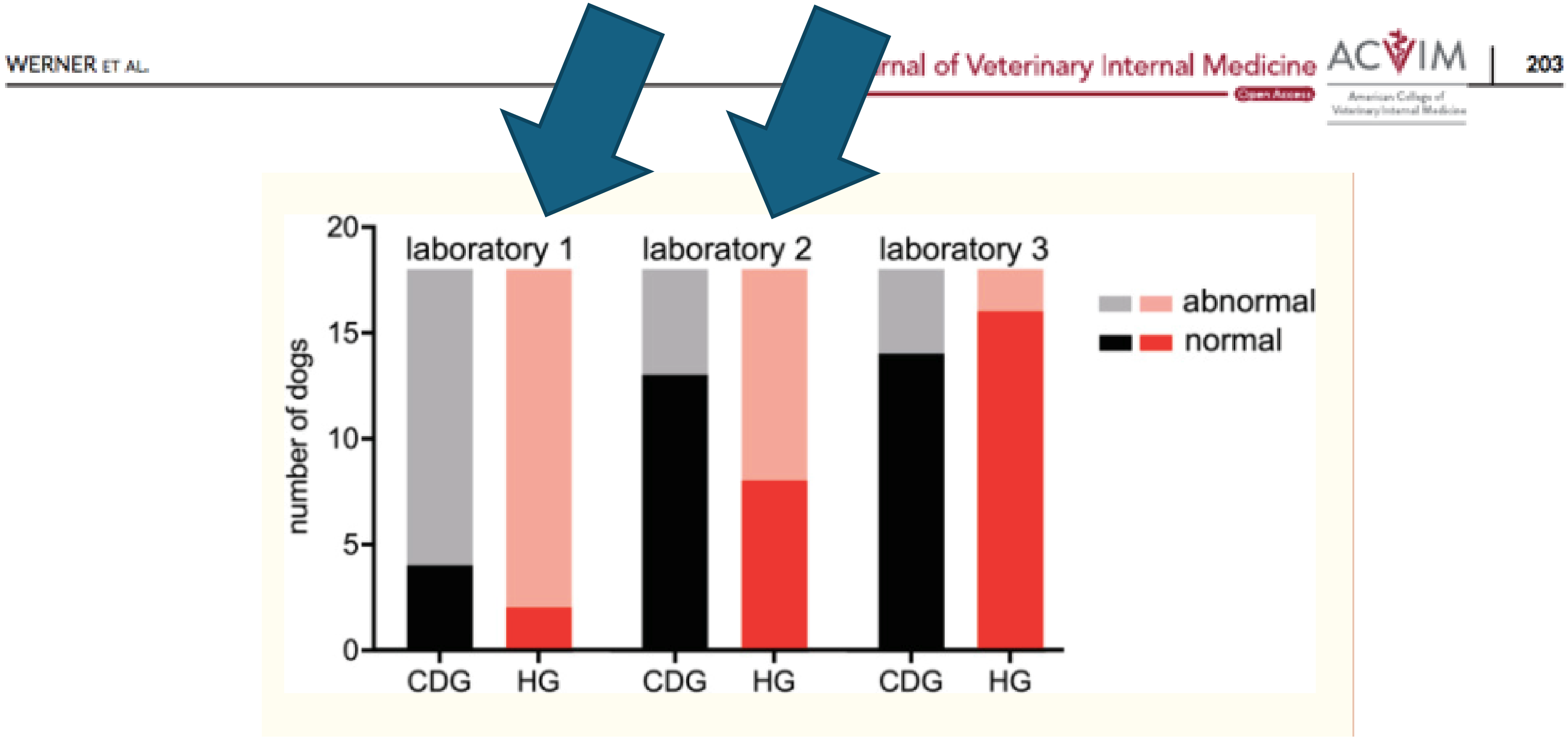
Diagnostic value of fecal cultures in dogs with chronic diarrhea

Melanie Werner[1] | Jan S. Suchodolski[2] | Jonathan A. Lidbury[2] |
Jörg M. Steiner[2] | Katrin Hartmann[1] | Stefan Unterer[1]

- Compared fecal culture 3 different anonymous vet labs with the dysbiosis index

- 18 dogs chronic diarrhea (CD) , 18 healthy dogs (H)

- Faecal cultures failed to distinguish between CD and H
- ***Clostridium spp*** – as prevalent in H as CD
- High level of interlaboratory variation for culture


WERNER ET AL.

rnal of Veterinary Internal Medicine ACVIM | 203

- 2/3 labs reported abnormal fecal microbiome, based on culture, in more healthy dogs than CD

- Growth of **hemolytic *E. coli*** could not differ CD from H

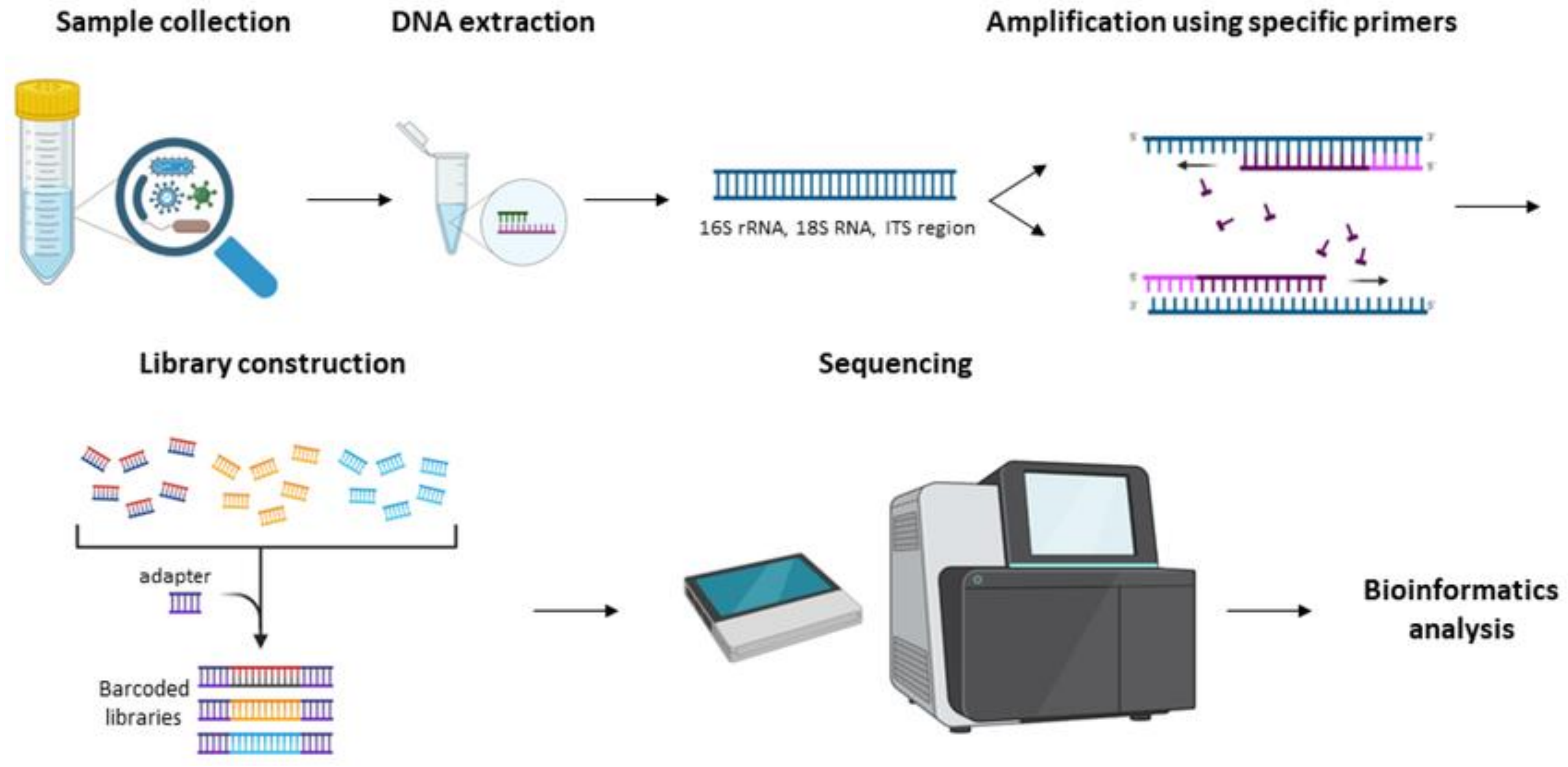
➔ Fecal cultures failed to distinguish between CD and H


WERNER ET AL.

Journal of Veterinary Internal Medicine Open Access ACVIM American College of Veterinary Internal Medicine | 203

- Dysbiosis index significantly higher in dogs w CD/H (p=0.0002)
- Green dots - decreased abundance of *Peptacetobacter hiranonis*
- Abundance of *Faecalibacterium* and *Fusobacterium* sign. lower in CD/H

Targeted amplicon sequencing - *16S rRNA gene*

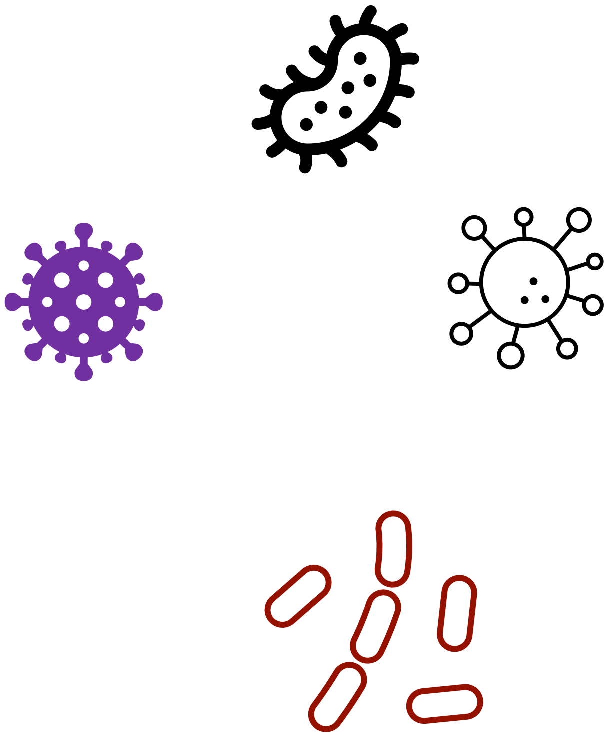


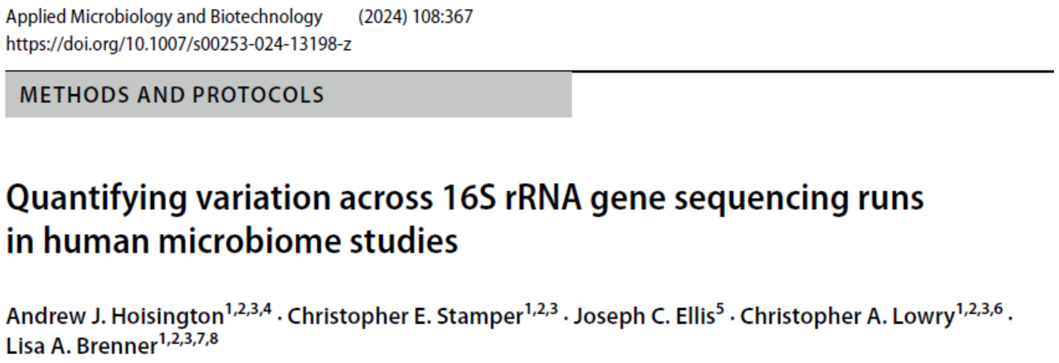
From ”Unveiling the Human Gastrointestinal Tract Microbiome: The Past, Present, and Future of Metagenomics” Athanasopoulou et al, Biomedicines 2023

Targeted amplicon sequencing - *16S rRNA gene*

- Pros
  - Rapid, non-culturable
  - Detection of many bacterial taxa simultaneously
    - identifies changes in bacterial communities

- Cons
  - Low taxonomic resolution on species level
    - mostly on genus level
  - only relative proportions of bacterial taxa reported
    - no absolute quantification
  - low reproducibility between runs
    - technology changes rapidly (results change)
    - no analytical validation reported
    - difficult to follow up patients over time





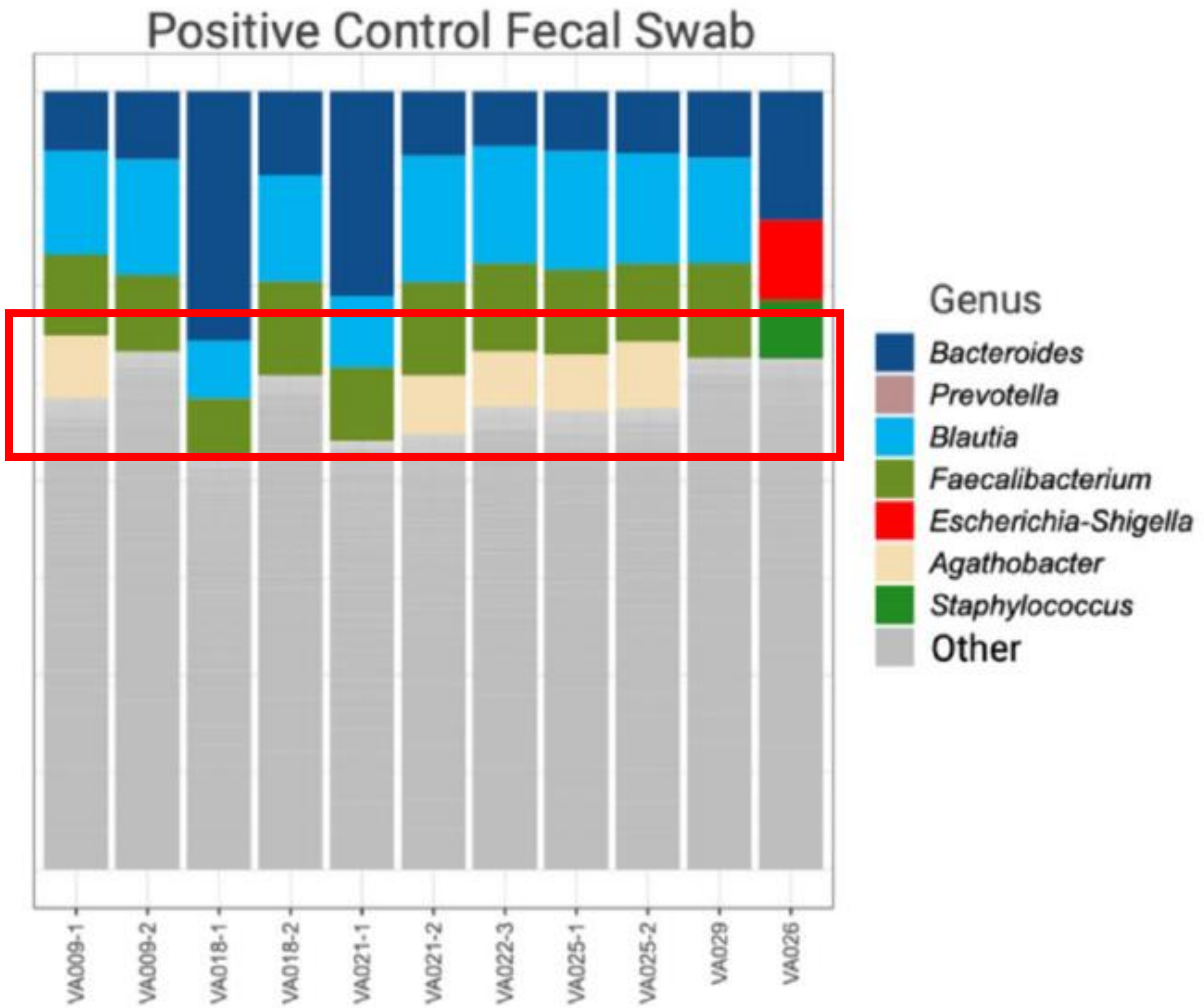
Applied Microbiology and Biotechnology (2024) 108:367
https://doi.org/10.1007/s00253-024-13198-z

METHODS AND PROTOCOLS

Quantifying variation across 16S rRNA gene sequencing runs in human microbiome studies

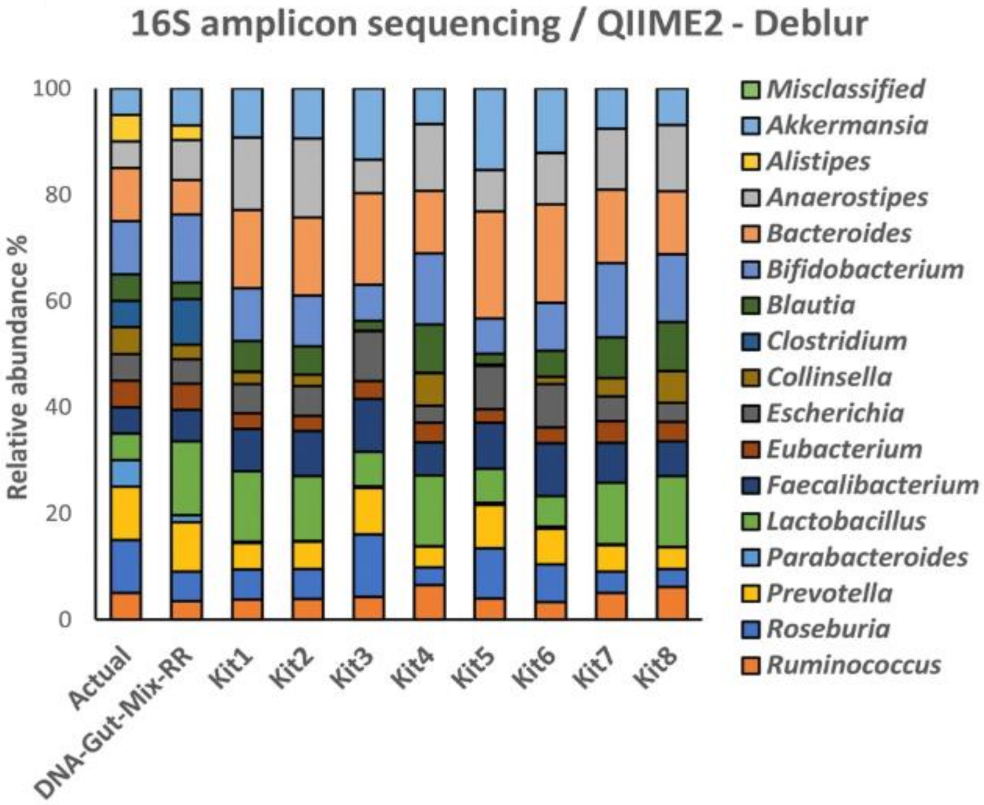
Andrew J. Hoisington[1,2,3,4] · Christopher E. Stamper[1,2,3] · Joseph C. Ellis[5] · Christopher A. Lowry[1,2,3,6] · Lisa A. Brenner[1,2,3,7,8]

- Standardized positive control- aliquots of the same sample analyzed multiple times at. The same lab
  - High intra- and interassay variability



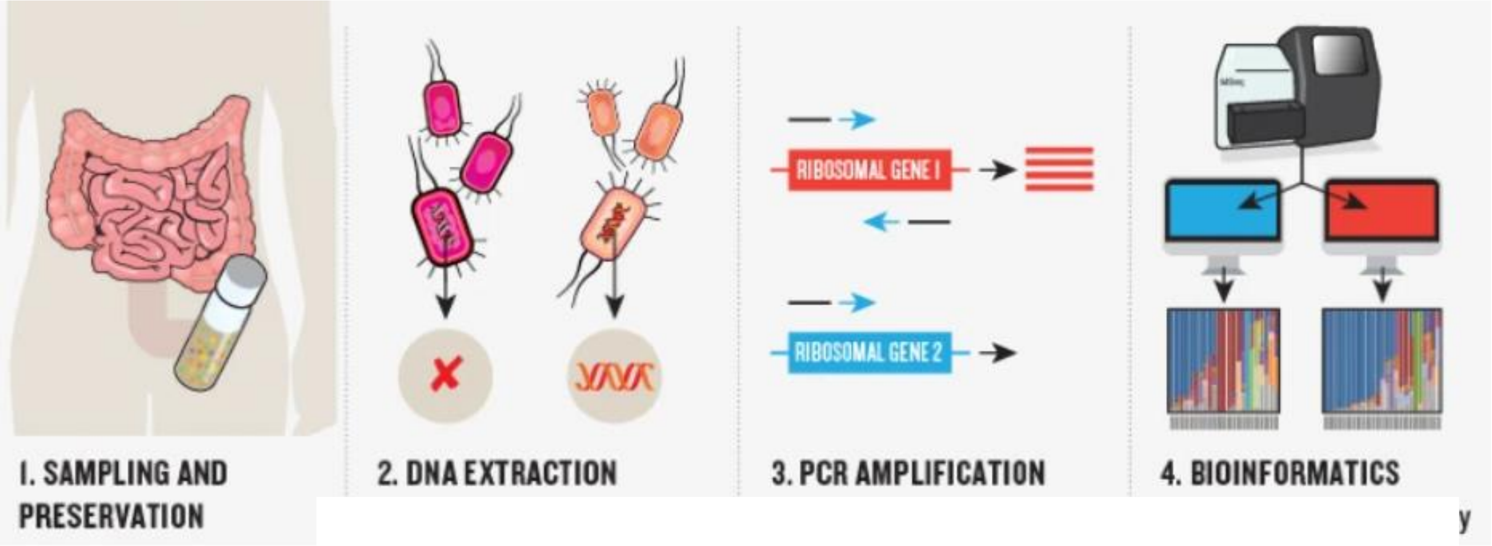
Positive Control Fecal Swab

Limited reproducibility

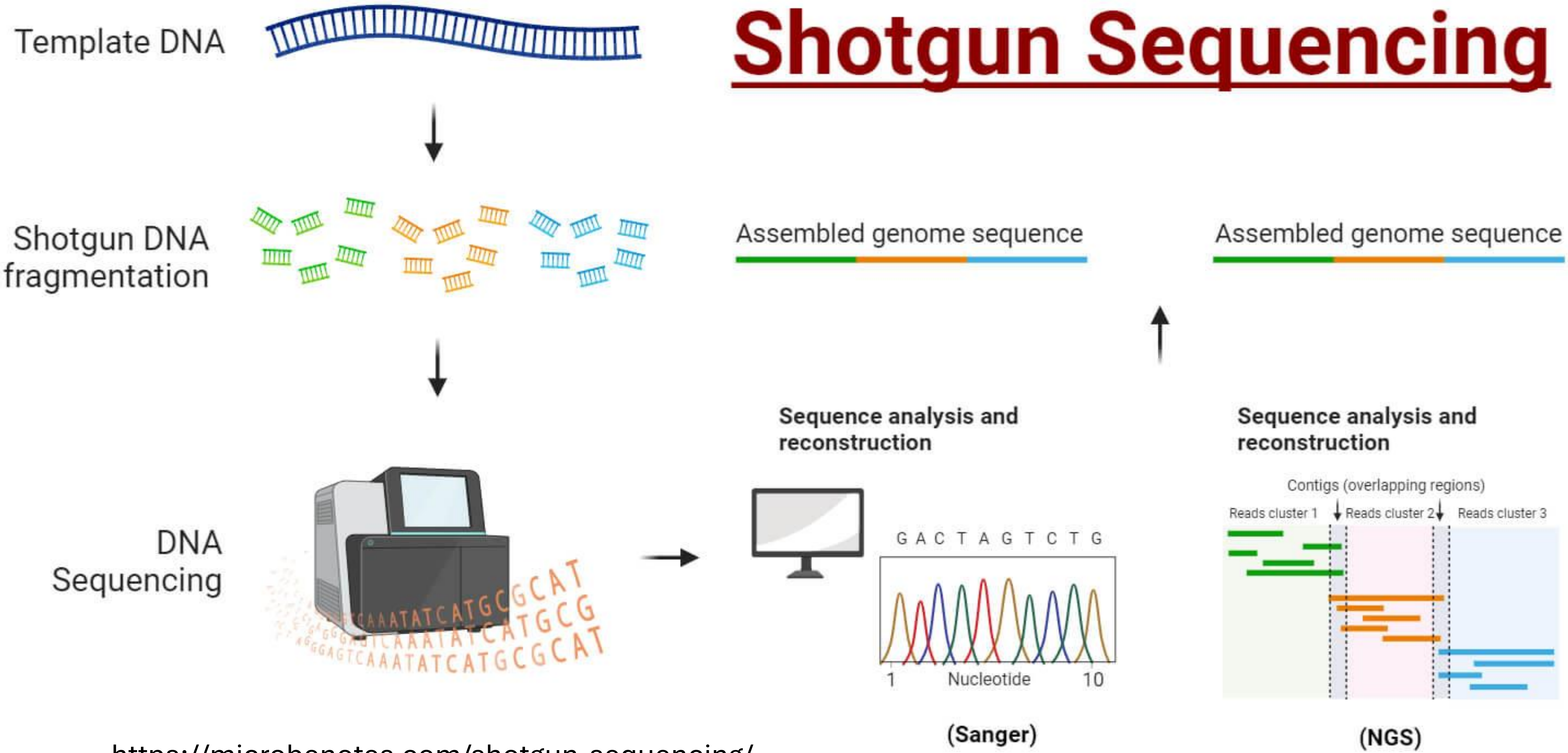


Sergaki et al, Microbiome 2022

Relatively **minor alterations** in the DNA extraction procedure or in the bioinformatics analysis can give a distorted view between studies

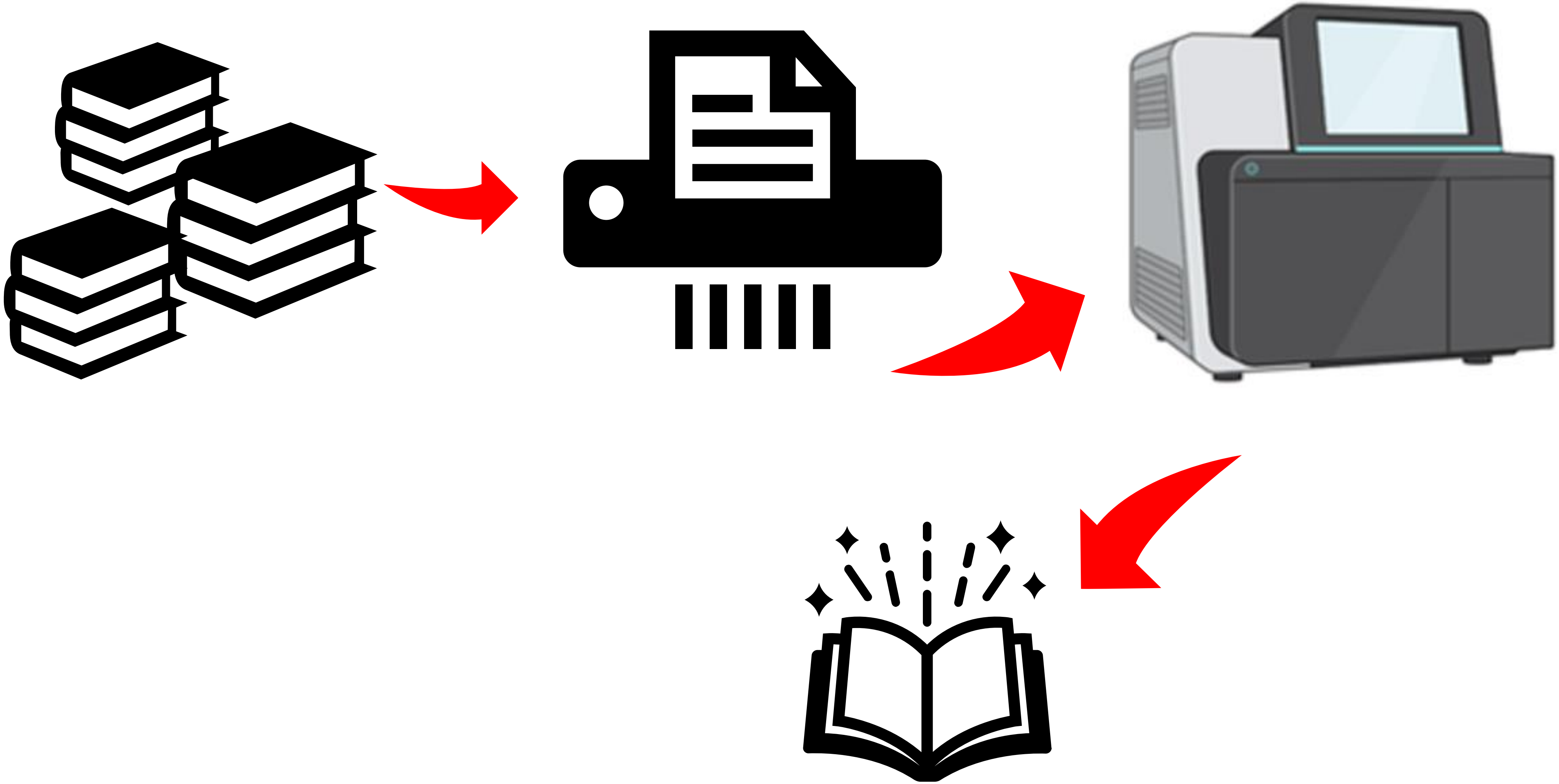


Shotgun Sequencing



https://microbenotes.com/shotgun-sequencing/

DNA Shotgun sequencing – if the microbiome was a book…



DNA Shotgun sequencing (Metagenomics)

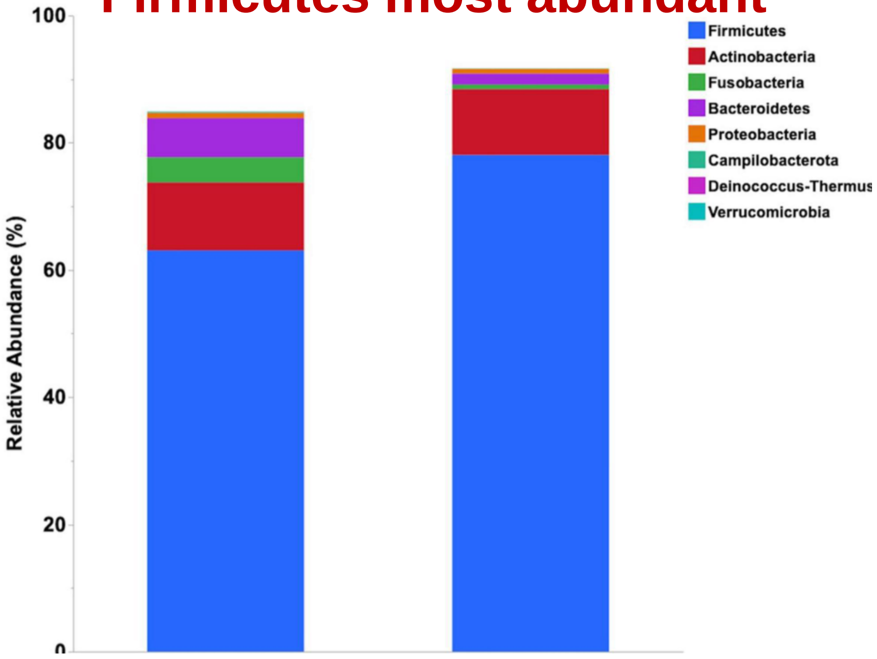
- Provides information about whole genome and microbial communities

- Pros
  - Functional (gene content) and identification of bacterial taxa
  - PCR independent (no bias)
  - Better resolution at species and strain level

- Cons
  - Higher cost per sample
  - Requiring very extensive computer processing power
  - Reports only relative proportions (no true quantification)

Major differences in reported taxonomy, even at baseline, for healthy dogs

Firmicutes most abundant



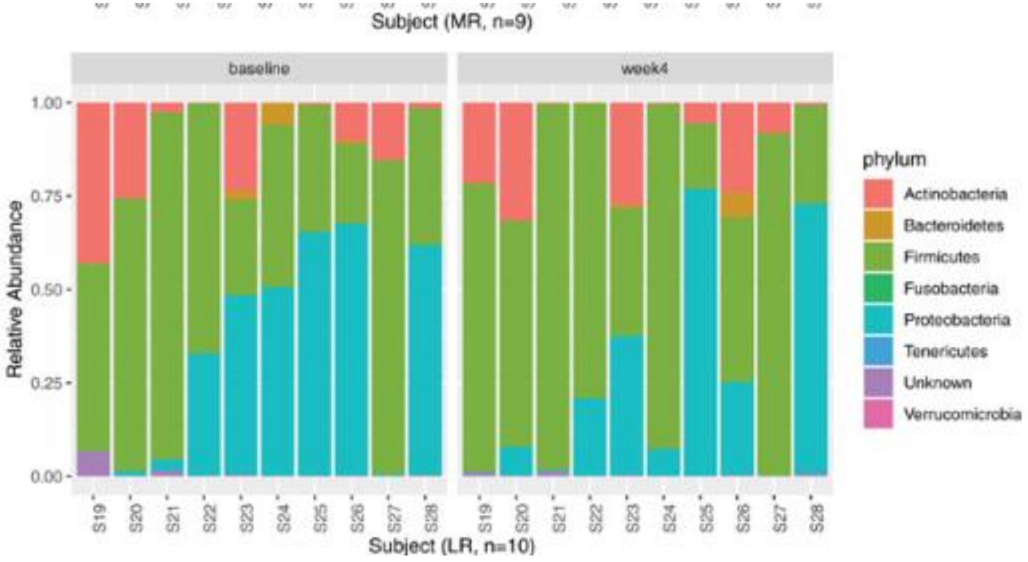
Liversidge BD et al, Front Microbiol 2024

Bacteroides most abundant

Allaway et al, AEM 2020

https://doi.org/10.1128/AEM.00562-20

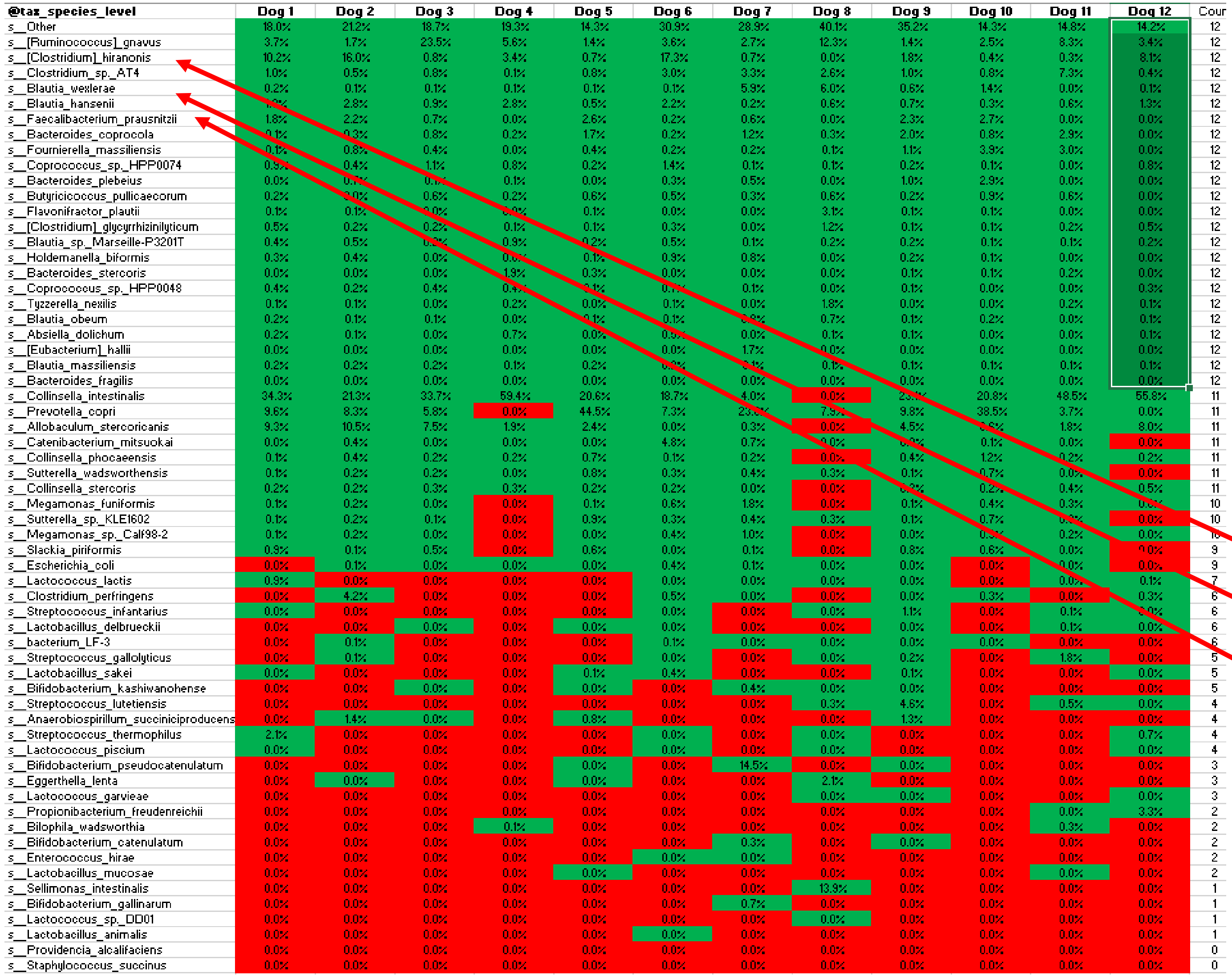
Proteobacteria most abundant



Tanprasertsuk et al, PeerJ 2021

https://pubmed.ncbi.nlm.nih.gov/34249503/

The canine/feline Core Microbiome - DNA shotgun sequencing



@taz_species_level	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6	Dog 7	Dog 8	Dog 9	Dog 10	Dog 11	Dog 12	Cour
s__Other	18.0%	21.2%	18.7%	19.3%	14.3%	30.9%	28.9%	40.1%	35.2%	14.3%	14.8%	14.2%	12
s__[Ruminococcus]_gnavus	3.7%	1.7%	23.5%	5.6%	1.4%	3.6%	2.7%	12.3%	1.4%	2.5%	8.3%	3.4%	12
s__[Clostridium]_hiranonis	10.2%	16.0%	0.8%	3.4%	0.7%	17.3%	0.7%	0.0%	1.8%	0.4%	0.3%	8.1%	12
s__Clostridium_sp._AT4	1.0%	0.5%	0.8%	0.1%	0.8%	3.0%	3.3%	2.6%	1.0%	0.8%	7.3%	0.4%	12
s__Blautia_wexlerae	0.2%	0.1%	0.1%	0.1%	0.1%	0.1%	5.9%	6.0%	0.6%	1.4%	0.0%	0.1%	12
s__Blautia_hansenii	[illegible]	2.8%	0.9%	2.8%	0.5%	2.2%	0.2%	0.6%	0.7%	0.3%	0.6%	1.3%	12
s__Faecalibacterium_prausnitzii	1.8%	2.2%	0.7%	0.0%	2.6%	0.2%	0.6%	0.0%	2.3%	2.7%	0.0%	0.0%	12
s__Bacteroides_coprocola	0.1%	0.3%	0.8%	0.2%	1.7%	0.2%	1.2%	0.3%	2.0%	0.8%	2.9%	0.0%	12
s__Fournierella_massiliensis	0.1%	0.8%	0.4%	0.0%	0.4%	0.2%	0.2%	0.1%	1.1%	3.9%	3.0%	0.0%	12
s__Coprococcus_sp._HPP0074	0.9%	0.4%	1.1%	0.8%	0.2%	1.4%	0.1%	0.1%	0.2%	0.1%	0.0%	0.8%	12
s__Bacteroides_plebeius	0.0%	0.1%	0.1%	0.1%	0.0%	0.3%	0.5%	0.0%	1.0%	2.9%	0.0%	0.0%	12
s__Butyricicoccus_pullicaecorum	0.2%	0.0%	0.6%	0.2%	0.6%	0.5%	0.3%	0.6%	0.2%	0.9%	0.6%	0.0%	12
s__Flavonifractor_plautii	0.1%	0.1%	0.0%	0.0%	0.1%	0.0%	0.0%	3.1%	0.1%	0.1%	0.0%	0.0%	12
s__[Clostridium]_glycyrrhizinilyticum	0.5%	0.2%	0.2%	0.1%	0.1%	0.3%	0.0%	1.2%	0.1%	0.1%	0.2%	0.5%	12
s__Blautia_sp._Marseille-P3201T	0.4%	0.5%	0.2%	0.9%	0.2%	0.5%	0.1%	0.2%	0.2%	0.1%	0.1%	0.2%	12
s__Holdemanella_biformis	0.3%	0.4%	0.0%	0.0%	0.1%	0.9%	0.8%	0.0%	0.2%	0.1%	0.0%	0.0%	12
s__Bacteroides_stercoris	0.0%	0.0%	0.0%	1.9%	0.3%	0.0%	0.0%	0.0%	0.1%	0.1%	0.2%	0.0%	12
s__Coprococcus_sp._HPP0048	0.4%	0.2%	0.4%	0.4%	0.1%	0.1%	0.1%	0.0%	0.1%	0.0%	0.0%	0.3%	12
s__Tyzzerella_nexilis	0.1%	0.1%	0.0%	0.2%	0.0%	0.1%	0.0%	1.8%	0.0%	0.0%	0.2%	0.1%	12
s__Blautia_obeum	0.2%	0.1%	0.1%	0.0%	0.1%	0.1%	0.0%	0.7%	0.1%	0.2%	0.0%	0.1%	12
s__Absiella_dolichum	0.2%	0.1%	0.0%	0.7%	0.0%	0.5%	0.0%	0.1%	0.1%	0.0%	0.0%	0.1%	12
s__[Eubacterium]_hallii	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.7%	0.0%	0.0%	0.0%	0.0%	0.0%	12
s__Blautia_massiliensis	0.2%	0.2%	0.2%	0.1%	0.2%	0.0%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	12
s__Bacteroides_fragilis	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	12
s__Collinsella_intestinalis	34.3%	21.3%	33.7%	59.4%	20.6%	18.7%	4.0%	0.0%	23.1%	20.8%	48.5%	55.8%	11
s__Prevotella_copri	9.6%	8.3%	5.8%	0.0%	44.5%	7.3%	23.0%	7.9%	9.8%	38.5%	3.7%	0.0%	11
s__Allobaculum_stercoricanis	9.3%	10.5%	7.5%	1.9%	2.4%	0.0%	0.3%	0.0%	4.5%	0.6%	1.8%	8.0%	11
s__Catenibacterium_mitsuokai	0.0%	0.4%	0.0%	0.0%	0.0%	4.8%	0.7%	0.0%	0.0%	0.1%	0.0%	0.0%	11
s__Collinsella_phocaeensis	0.1%	0.4%	0.2%	0.2%	0.7%	0.1%	0.2%	0.0%	0.4%	1.2%	0.2%	0.2%	11
s__Sutterella_wadsworthensis	0.1%	0.2%	0.2%	0.0%	0.8%	0.3%	0.4%	0.3%	0.1%	0.7%	0.0%	0.0%	11
s__Collinsella_stercoris	0.2%	0.2%	0.3%	0.3%	0.2%	0.2%	0.0%	0.0%	0.2%	0.2%	0.4%	0.5%	11
s__Megamonas_funiformis	0.1%	0.2%	0.0%	0.0%	0.1%	0.6%	1.8%	0.0%	0.1%	0.4%	0.3%	0.0%	10
s__Sutterella_sp._KLE1602	0.1%	0.2%	0.1%	0.0%	0.9%	0.3%	0.4%	0.3%	0.1%	0.7%	0.0%	0.0%	10
s__Megamonas_sp._Calf98-2	0.1%	0.2%	0.0%	0.0%	0.0%	0.4%	1.0%	0.0%	0.0%	0.3%	0.2%	0.0%	10
s__Slackia_piriformis	0.9%	0.1%	0.5%	0.0%	0.6%	0.0%	0.1%	0.0%	0.8%	0.6%	0.0%	0.0%	9
s__Escherichia_coli	0.0%	0.1%	0.0%	0.0%	0.0%	0.4%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	9
s__Lactococcus_lactis	0.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	7
s__Clostridium_perfringens	0.0%	4.2%	0.0%	0.0%	0.0%	0.5%	0.0%	0.0%	0.0%	0.3%	0.0%	0.3%	6
s__Streptococcus_infantarius	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.1%	0.0%	0.1%	0.0%	6
s__Lactobacillus_delbrueckii	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	6
s__bacterium_LF-3	0.0%	0.1%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	6
s__Streptococcus_gallolyticus	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	1.8%	0.0%	5
s__Lactobacillus_sakei	0.0%	0.0%	0.0%	0.0%	0.1%	0.4%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	5
s__Bifidobacterium_kashiwanohense	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.4%	0.0%	0.0%	0.0%	0.0%	0.0%	5
s__Streptococcus_lutetiensis	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	4.6%	0.0%	0.5%	0.0%	4
s__Anaerobiospirillum_succiniciproducens	0.0%	1.4%	0.0%	0.0%	0.8%	0.0%	0.0%	0.0%	1.3%	0.0%	0.0%	0.0%	4
s__Streptococcus_thermophilus	2.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.7%	4
s__Lactococcus_piscium	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	4
s__Bifidobacterium_pseudocatenulatum	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	14.5%	0.0%	0.0%	0.0%	0.0%	0.0%	3
s__Eggerthella_lenta	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.1%	0.0%	0.0%	0.0%	0.0%	3
s__Lactococcus_garvieae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	3
s__Propionibacterium_freudenreichii	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	3.3%	2
s__Bilophila_wadsworthia	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	0.0%	2
s__Bifidobacterium_catenulatum	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	2
s__Enterococcus_hirae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2
s__Lactobacillus_mucosae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2
s__Sellimonas_intestinalis	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	13.9%	0.0%	0.0%	0.0%	0.0%	1
s__Bifidobacterium_gallinarum	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.7%	0.0%	0.0%	0.0%	0.0%	0.0%	1
s__Lactococcus_sp._DD01	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1
s__Lactobacillus_animalis	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1
s__Providencia_alcalifaciens	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0
s__Staphylococcus_succinus	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0

- 1.065 bacteria species identified across 12 healthy dogs

- 25 identified in all 12/12 dogs
- 36 identified in 9/12 dogs

- Top core bacterial species and genera include
  - *Peptacetobacter hiranonis*
  - *Blautia*
  - *Faecalibacterium*

Quantitative PCR (DI)

- Pros
  - Quantitative: absolute abundance
  - Target selection: taxonomic and functional
  - <u>Highly reproducible across studies</u>
    - if same DNA extraction and PCR protocol used
  - <u>Allows establishment of reference intervals for specific taxa</u>

- Cons
  - Specific to the primers selected
  - Limited number of primers

Dysbios Index in dogs with CE - PCR based (7 bact groups + total)

CE vs healthy:

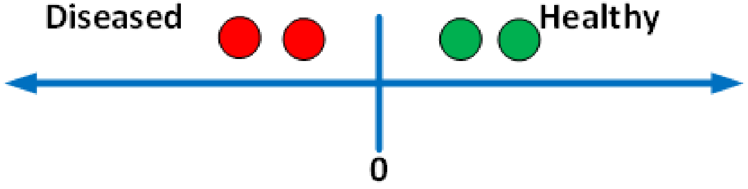
- Total amount bact. ↓
- *Faecalibacterium* ↑↑↑ ↑ ↓
- *Turicibacter* ↓
- *E.coli* ↑
- *Streptococcus* ↑
- *Blautia* ↓
- *Fusobacterium* ↓
- *C. hiranonis* ↓

$$L_2\text{:}\ \sqrt{\sum_{i=1}^{M}\left(z_i-\overline{y}_i'\right)^2}\ \overset{D}{\underset{H}{\lessgtr}}\ \sqrt{\sum_{i=1}^{M}\left(z_i-\overline{x}_i'\right)^2}\ \Leftrightarrow\ \Phi\ \overset{D}{\underset{H}{\lessgtr}}\ \Theta$$

Where $\Phi \equiv \sqrt{\Sigma_{i=1}^{M}\left(z_i-\overline{y}_i'\right)^2}$ *and* $\Theta \equiv \sqrt{\Sigma_{i=1}^{M}\left(z_i-\overline{x}_i'\right)^2}$

$$\therefore\ \ \Phi-\Theta\ \overset{D}{\underset{H}{\lessgtr}}\ 0 \Leftrightarrow \begin{cases} Diseased \Rightarrow (\Phi-\Theta)<0 \\ Healthy \Rightarrow (\Phi-\Theta)>0 \end{cases}$$

Therefore, the desired index $=(\Phi-\Theta)$.

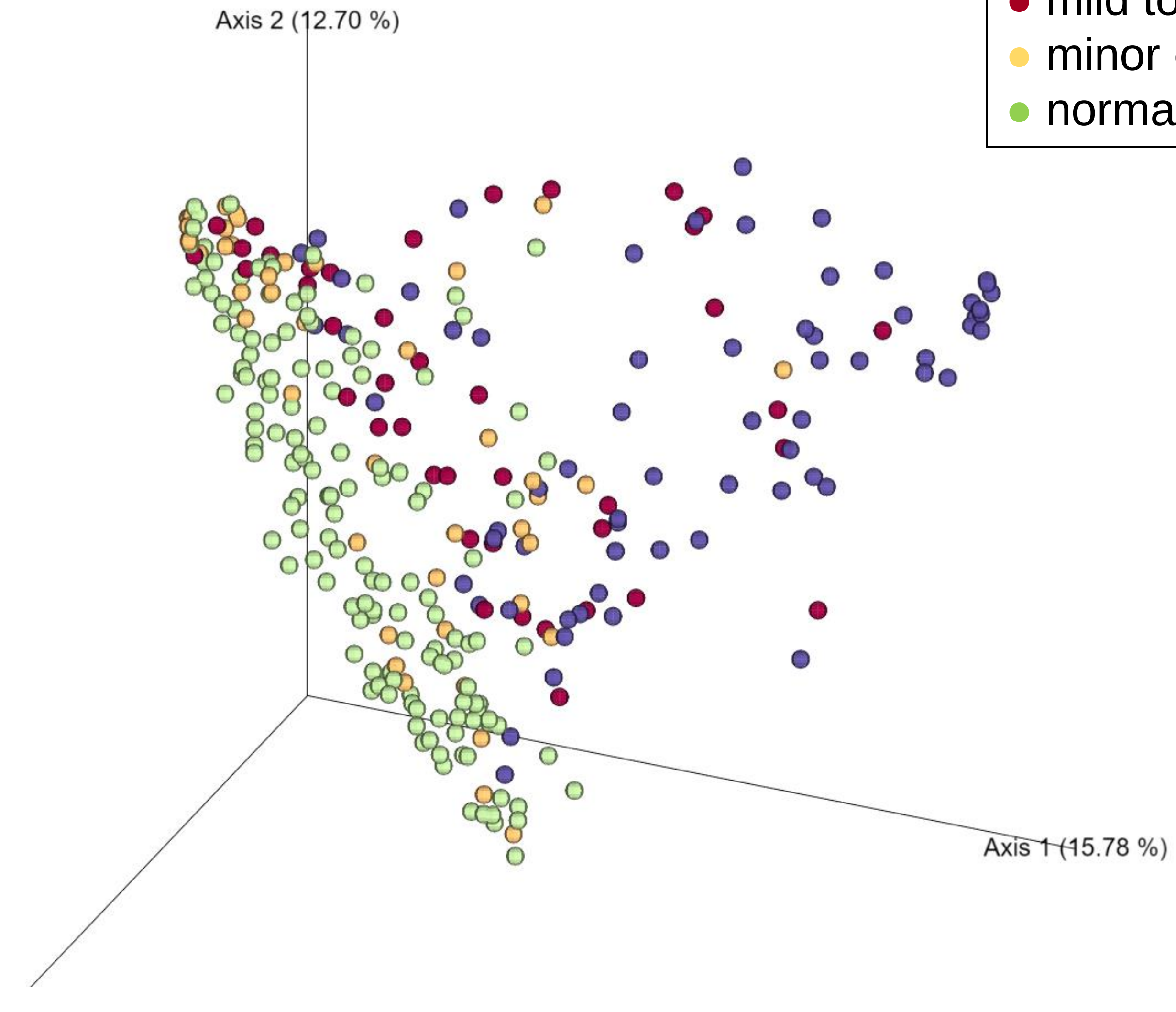


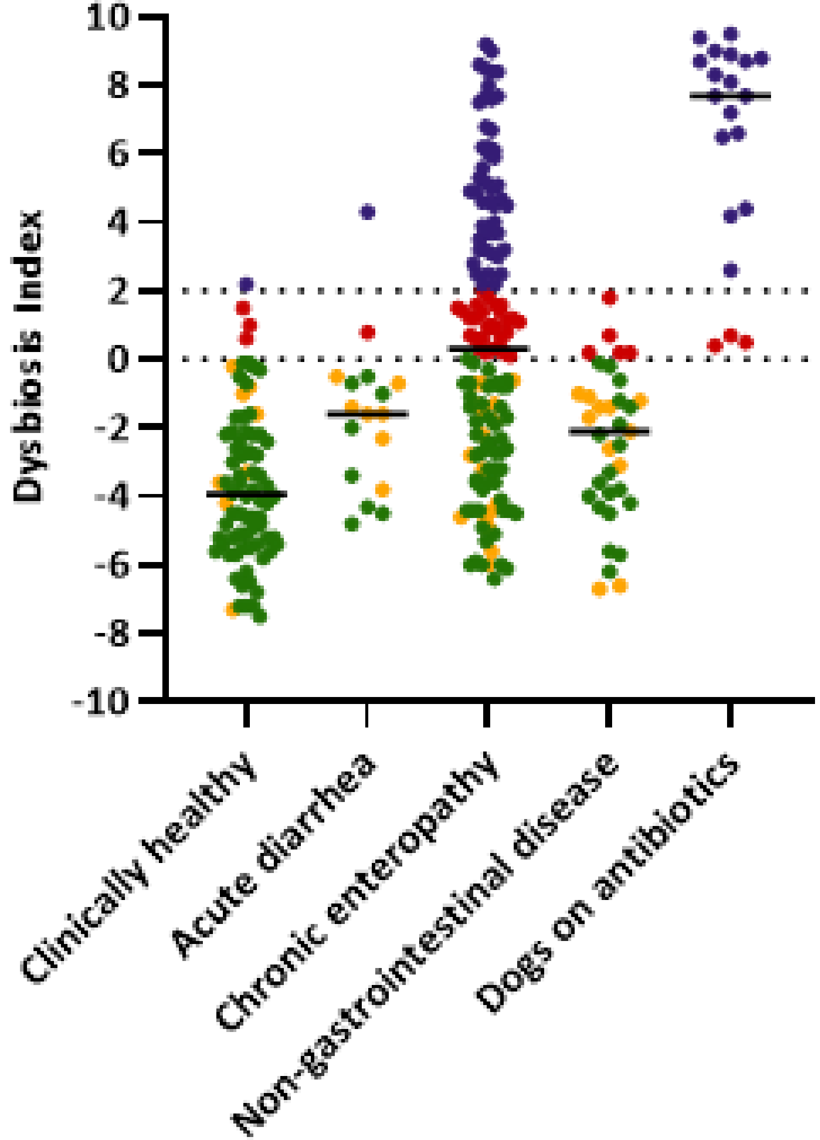
A dysbiosis index to assess microbial changes in fecal samples of dogs with chronic inflammatory enteropathy

FEMS Microbiology Ecology, 93, 2017,

MK AlShawaqfeh[1,2], B Wajid[1,3], Y Minamoto[1], M Markel[1], JA Lidbury[1], JM Steiner[1], E Serpedin[2] and JS Suchodolski[1,*]

- ● significant dysbiosis (DI > 2)
- ● mild to moderate changes (DI between 0-2)
- ● minor changes (DI < 0, some taxa abnormal)
- ● normal (DI < 0, all taxa normal)

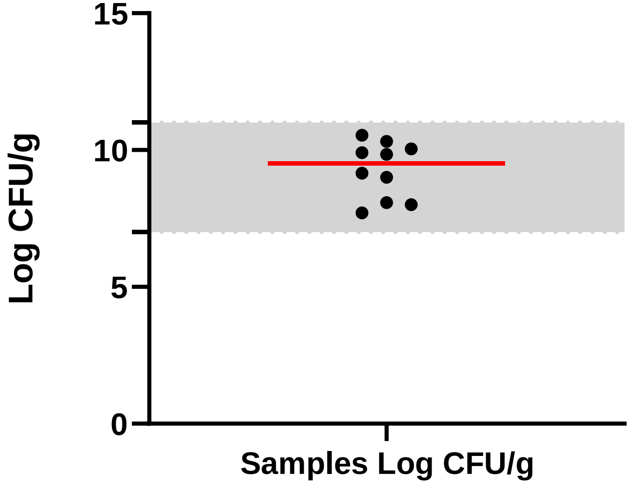


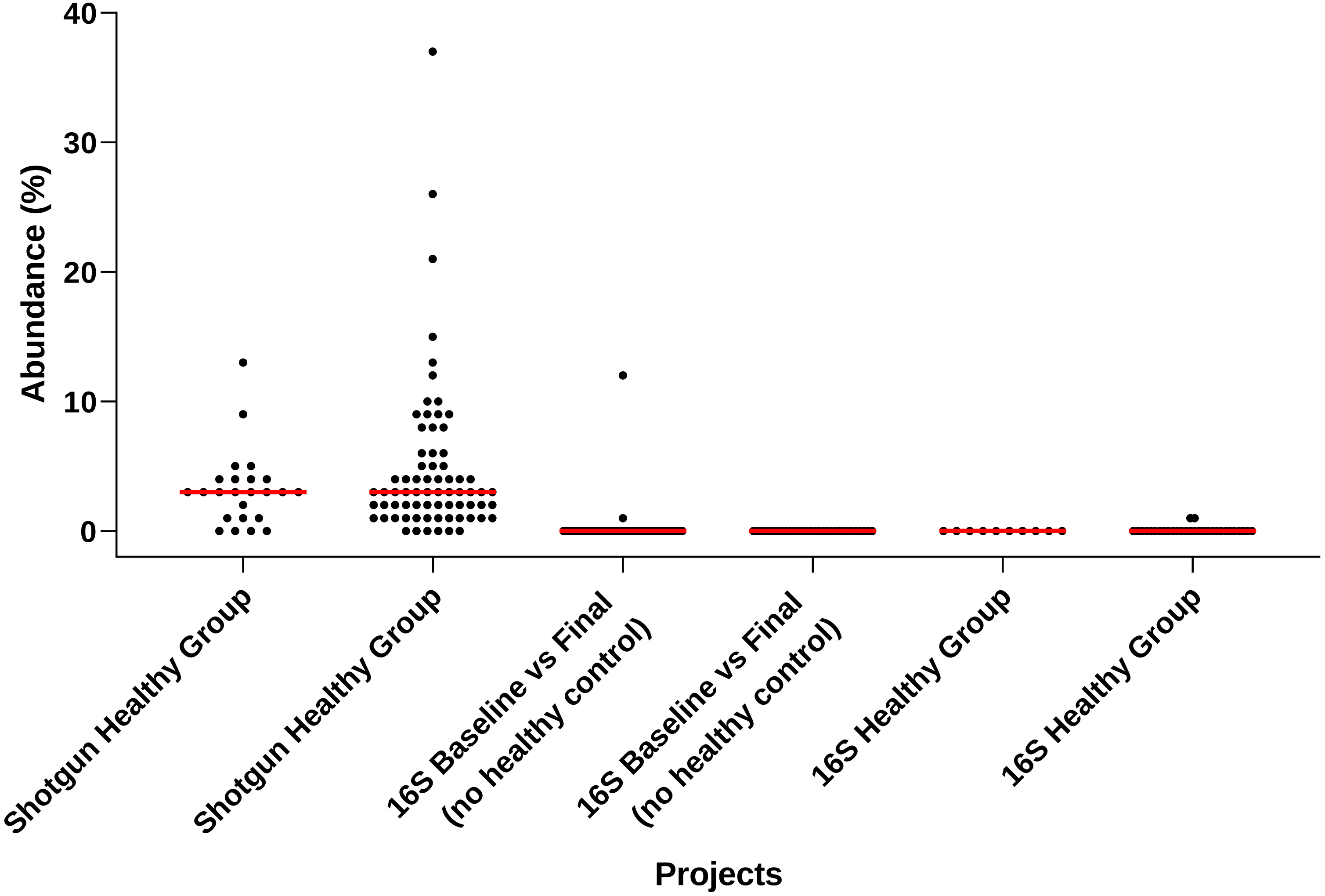


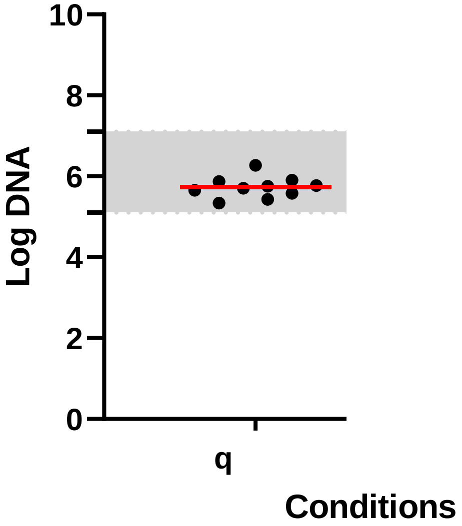
- Dysbiosis Index reflects microbiota shifts

Sung et al, Animals 2023

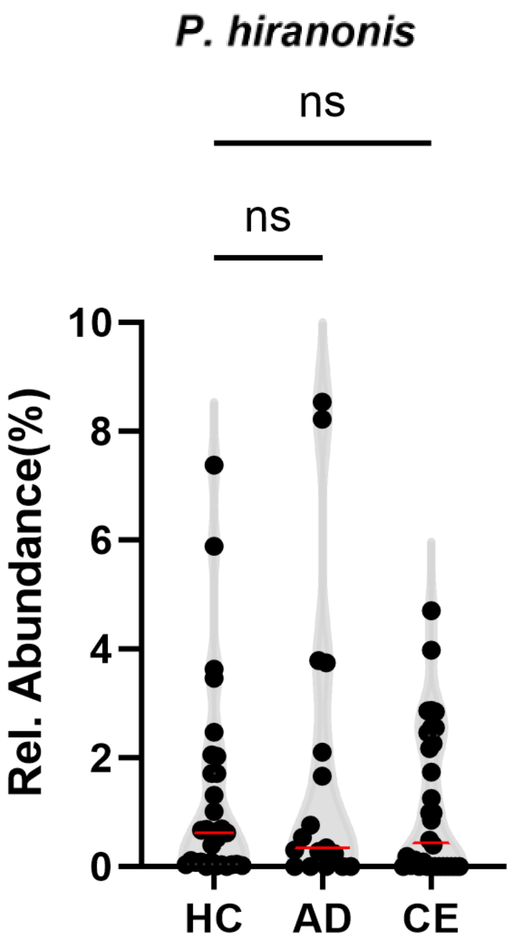
P. hiranonis abundance in feces – often undetected on sequencing


C. hiranonis **Viability in Fresh Feces (Log CFU/g)**

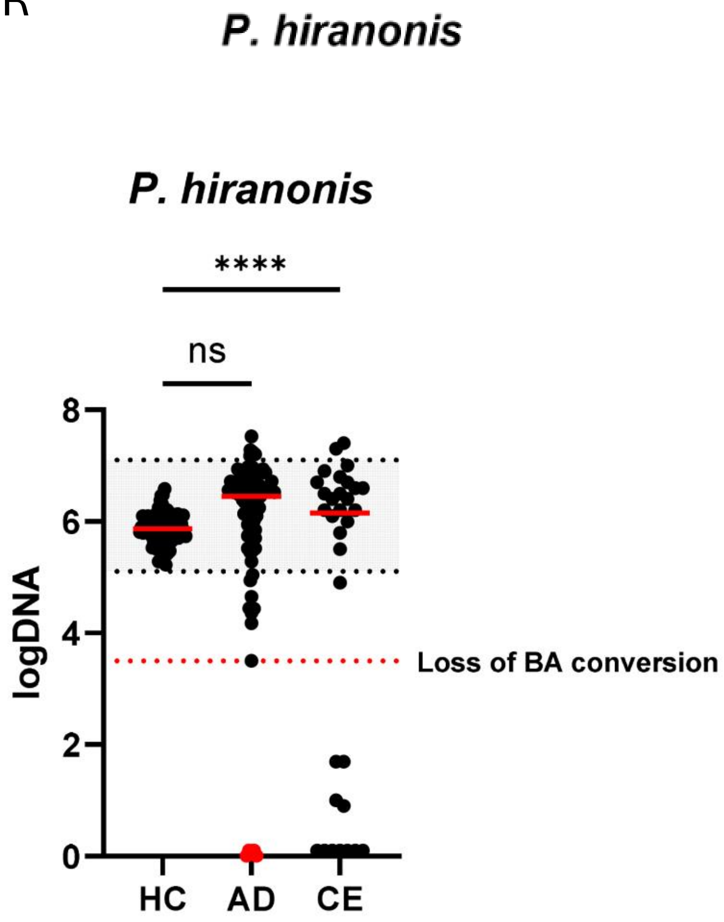

C. hiranonis **Relative Abundance in Sequencing**


C. hiranonis **in Fresh Feces (Log DNA)**

Sequencing



qPCR



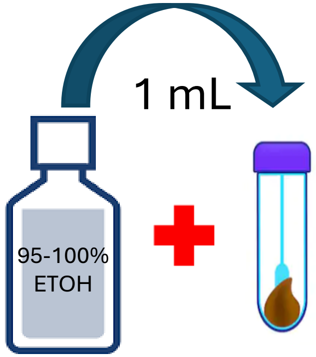
2 Options for analysis of Dysbiosis index (DI)

IDEXX
(samples re-directed to the GI lab, TAMU)

GI lab at
Texas A&M
University

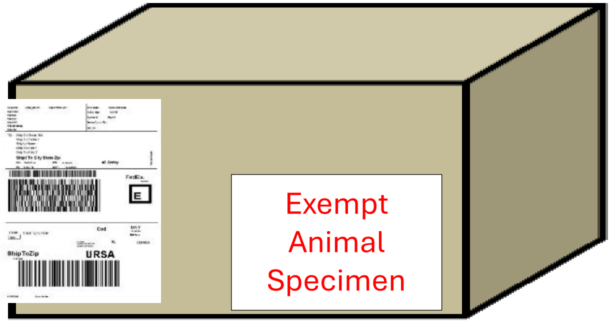
Ethanol Suspension

Product Info: 95 – 100% ETOH (any manufacturer)



Suspension Ratio: 1 mL ETOH : 0.5 g feces

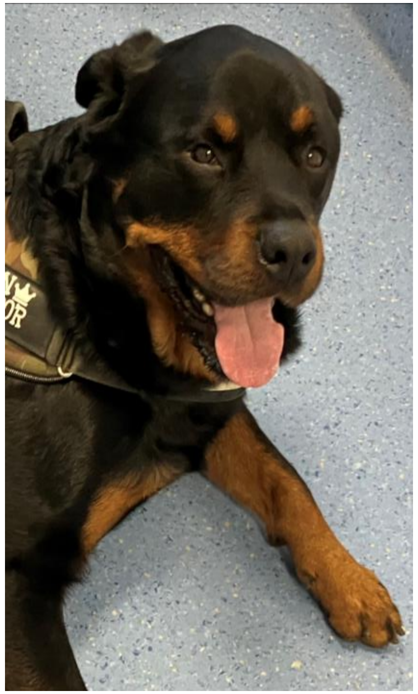
❖ Use 1:2 ratio of feces : ETOH
Total ETOH volume in your shipment *must not exceed 100 mL*



If shipping directly to the GI lab – contact them first for shipping instructions

Email: **gilab@tamu.edu**

”Tyson”, intact male Rottweiler, 4.5 Y



Referral, partially steroid- refractory PLE

- Liquid diarrhea 5-6 times/24 hrs since 4 months
- Lethargy, hyporexia, weight loss (12%)

Tx with cobalamin + corticosteroids for 60 days, tried hydrolyzed diet

and high fiber diet

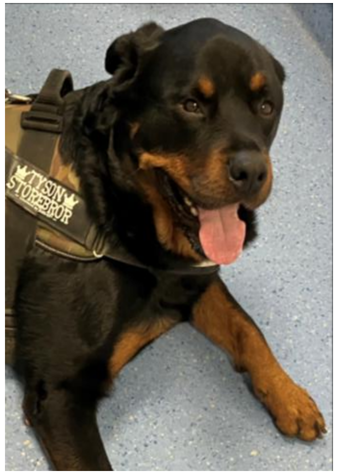
Suffers badly from corticosteroid side effects

- Panhypoproteinemia (TP 37 g/L), hypoalbuminemia (18 g/L), hypocholesterolemia (1.6 mmol/L)

- Low-fat hydrolyzed salmon-based diet, rivaroxabam + mirtazapin

"Tyson", intact male Rottweiler, 4.5 Y

- Histopathology report– moderate LP inflammation, mild lymphangiectasia
- No improvement of diarrhea/albumin → chlorambucil



- 3 w later: alb 23 g/L - still diarrhea

Dysbiosis index: **-4.3**

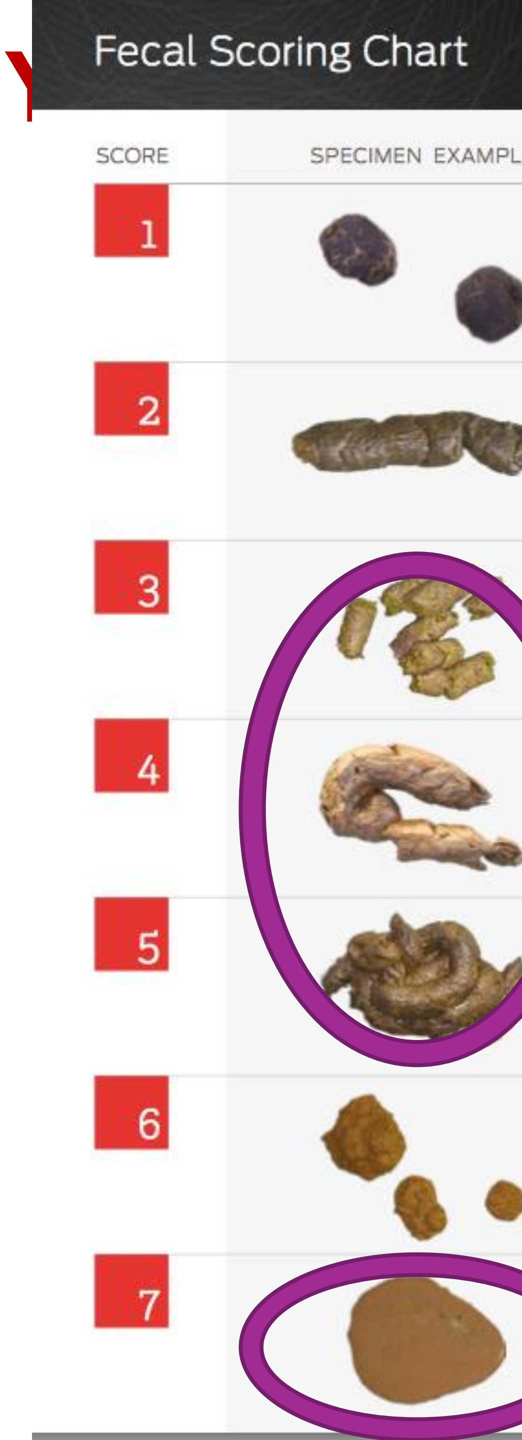
FMT– 3 treatments; rectal enema, 10- 20 days interval between tx

- After FMT 1 – stopped being fecally incontinent, freq. of defecation mildly
- After FMT 2 – BW increased 0.8 kg, more active, started playing, fecal score 5/7, freq. 3-5/24 hrs
- After FMT 3 – back to his normal self, plays a lot, + 2 kg, fecal score 3-5/7

”Tyson”, intact male Rottweiler, 5.3

- Doing very well, plays a lot with the younger dog, normal appetite, no side effects of meds

- Defecates x 4-5
- BCS 5/9, normal muscle status
- Serum biochemistry WNL
- Budesonide 3 mg/q 24 hrs, Chlorambucil 4 mg q 24 hrs – under tapering



veterinary sciences

MDPI

Article

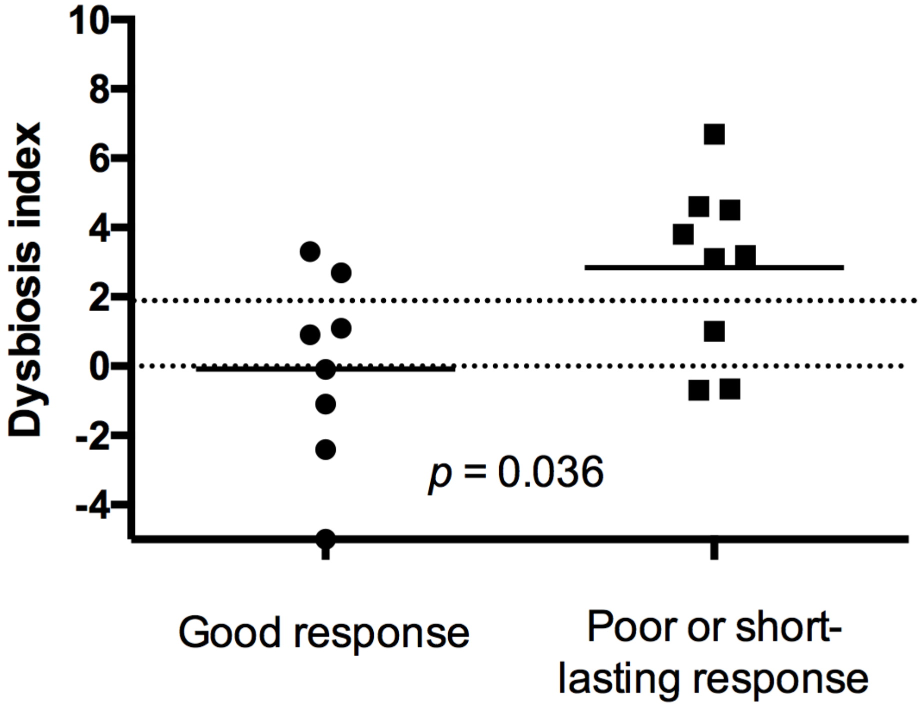
Clinical Effects of Faecal Microbiota Transplantation as Adjunctive Therapy in Dogs with Chronic Enteropathies—A Retrospective Case Series of 41 Dogs

Linda Toresson [1,2,*], Thomas Spillmann [1], Rachel Pilla [3], Ulrika Ludvigsson [2], Josefin Hellgren [2], Gunilla Olmedal [2] and Jan S. Suchodolski [3]

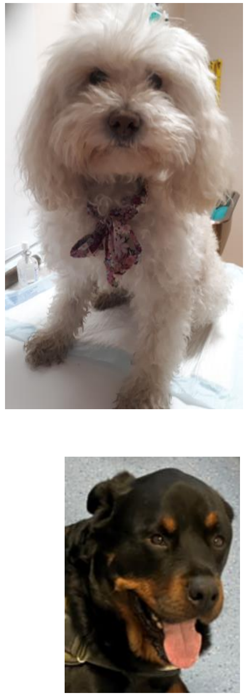
- Retrospective study, n=41

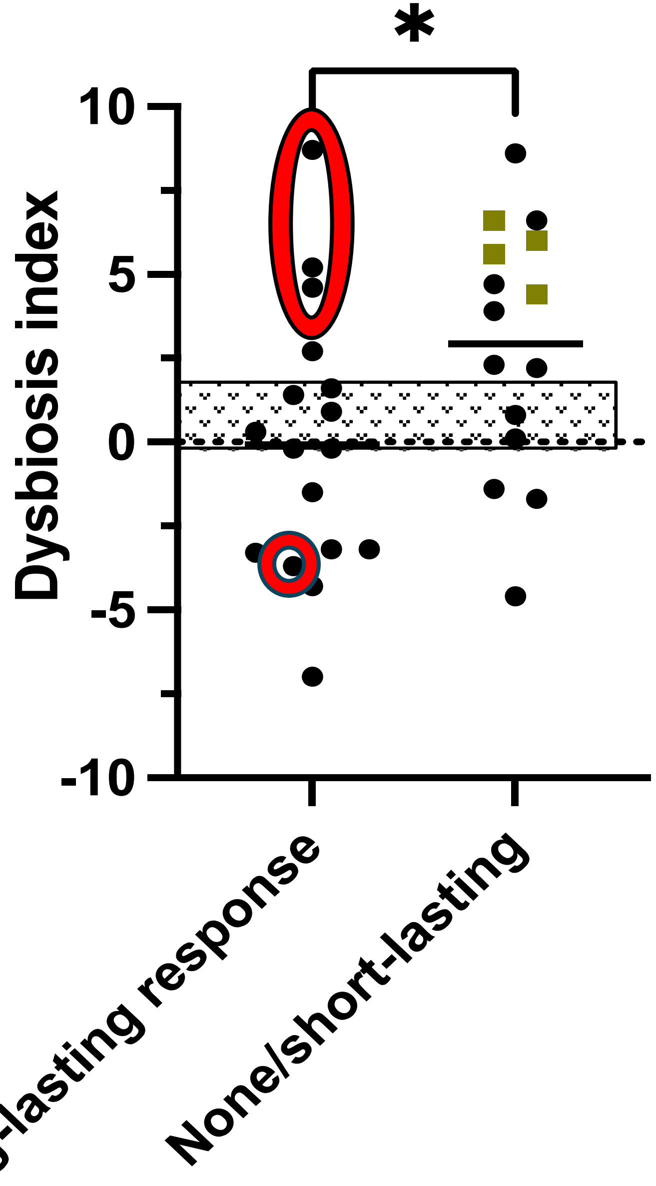
- 31/41 dogs (76%) responded to FMT

Dogs with a long-lasting response to FMT had lower DI at inclusion compared to non-responders or short-lasting responders



Preliminary results – prospective FMT study
DI at baseline



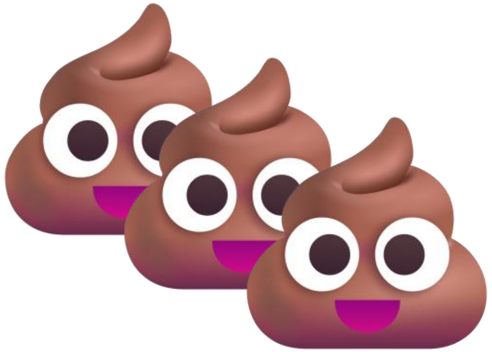


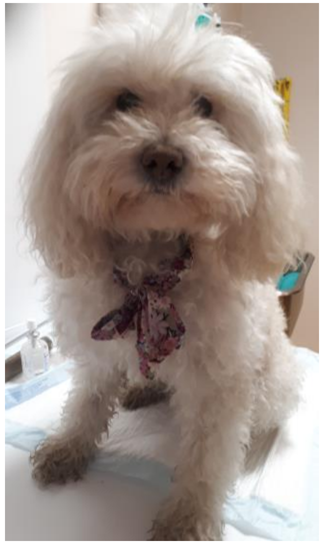
Dogs tx with antibiotics within 6 w. excluded

🟩 =responds to BA sequestrants

	Long-lasting	Non or short-lasting
Normobiosis	**9/17 (53%)**	**3/15 (20%)**
Mild dysbiosis	**4/17 (23.5 %)**	**2/15 (13%)**
Severe dysbiosis	**4/17 (23.5)%**	**10/15 (67%)**

”Kajsa”, intact female medium-sized poodle, 11 Y



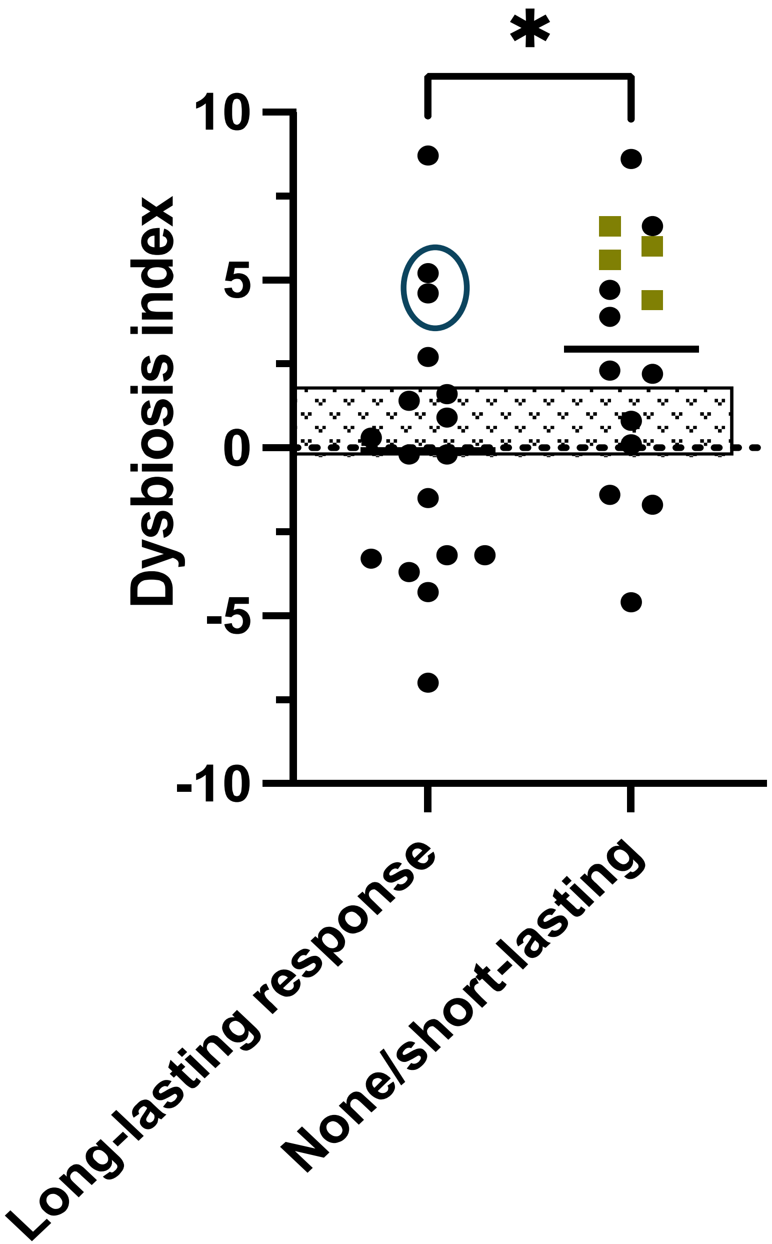


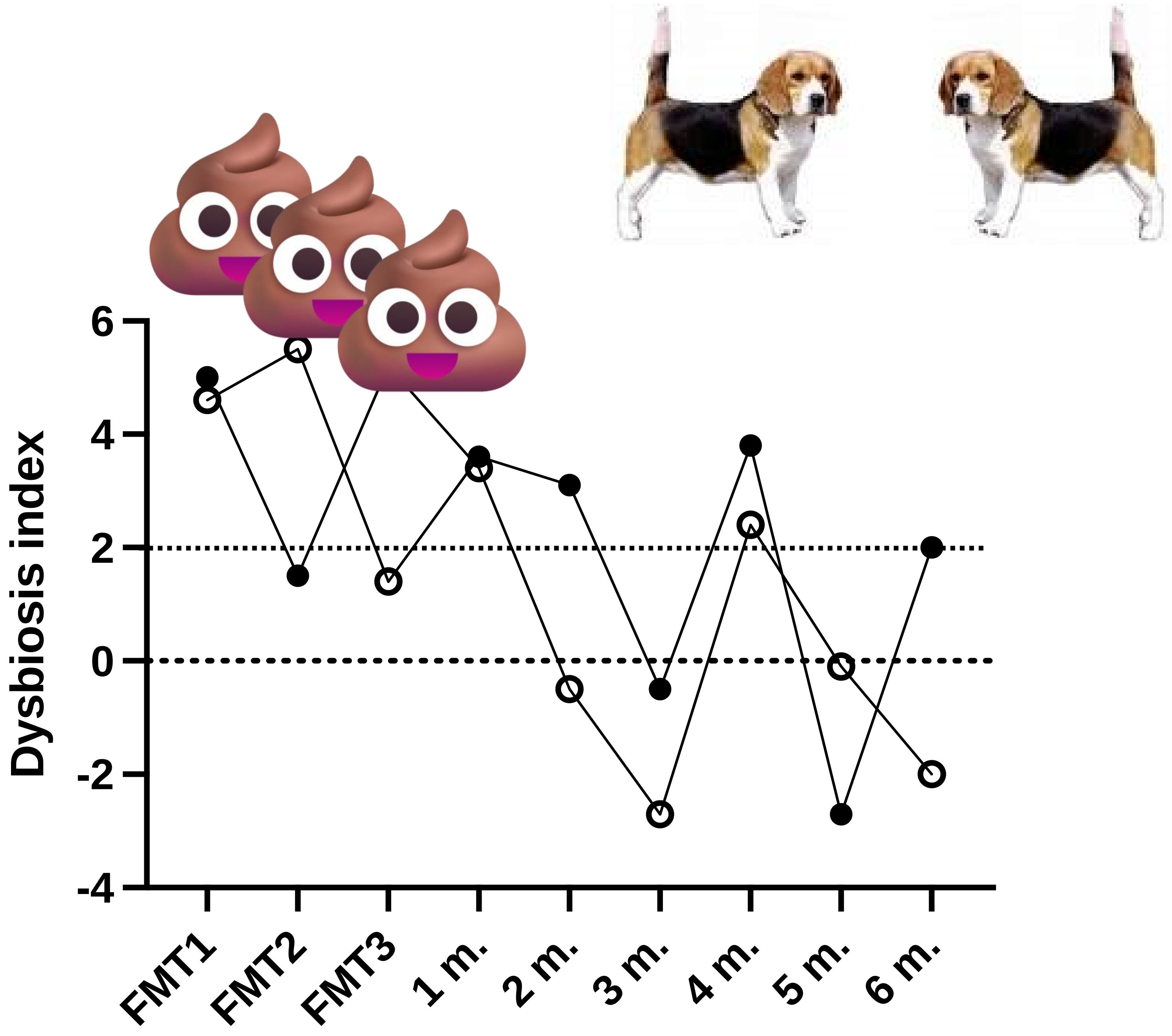
Tx for CE for 4 years w:

- Mono protein/hydrolyzed diet
- Pred q 48 hrs
- Olsalazine (5-ASA) q 48 hrs

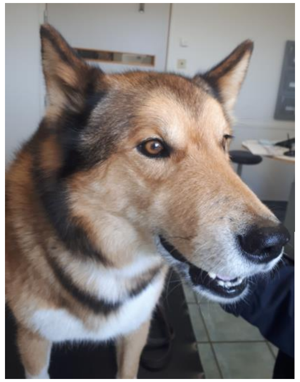
- Diarrhea for 2 months, not responding to diet change.

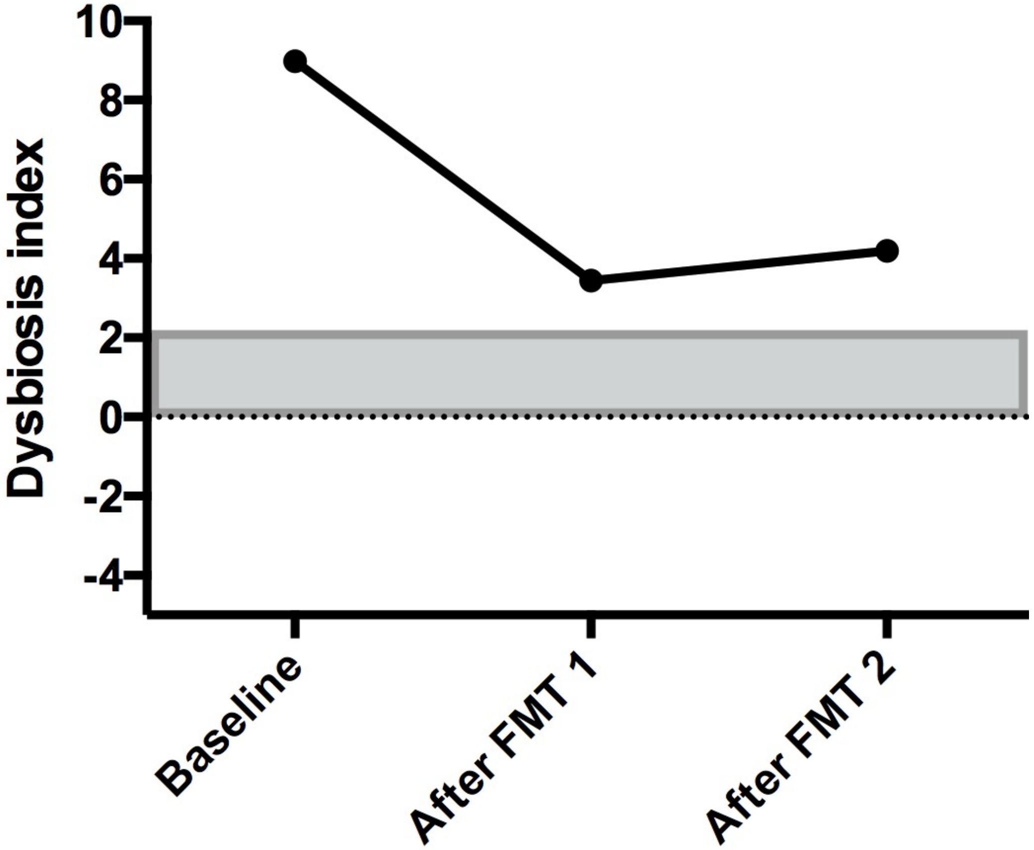
- Partial response to ⇧ Pred


*
Dysbiosis index
10
5
0
-5
-10
Long-lasting response
None/short-lasting


Dysbiosis index
6
4
2
0
-2
-4
FMT1
FMT2
FMT3
1 m.
2 m.
3 m.
4 m.
5 m.
6 m.

"Jurij", intact East Siberian Laika 10 Y/A, NRE





Main complaint: refractory diarrhea
Immediate clinical improvement post FMT

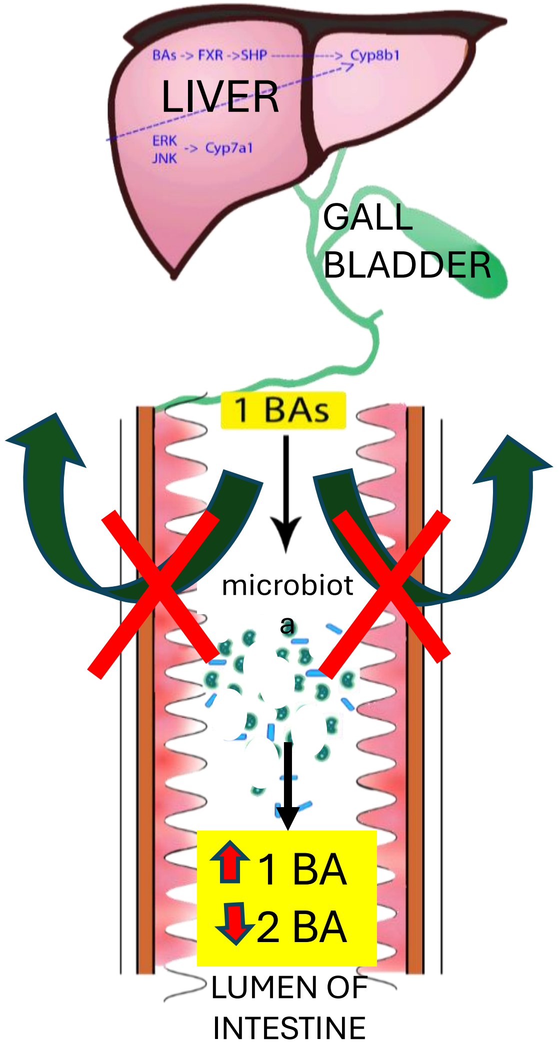
Relapse within 4 weeks post FMT 3

BAR, normal BCS, but diarrhea every week

Persistently low *Clostridium hiranonis* - increased risk of bile acid diarrhea (BAD)

Started cholestyramine (bile acid sequestrant) – diarrhea stopped

Bile Acid Diarrhea (BAD)



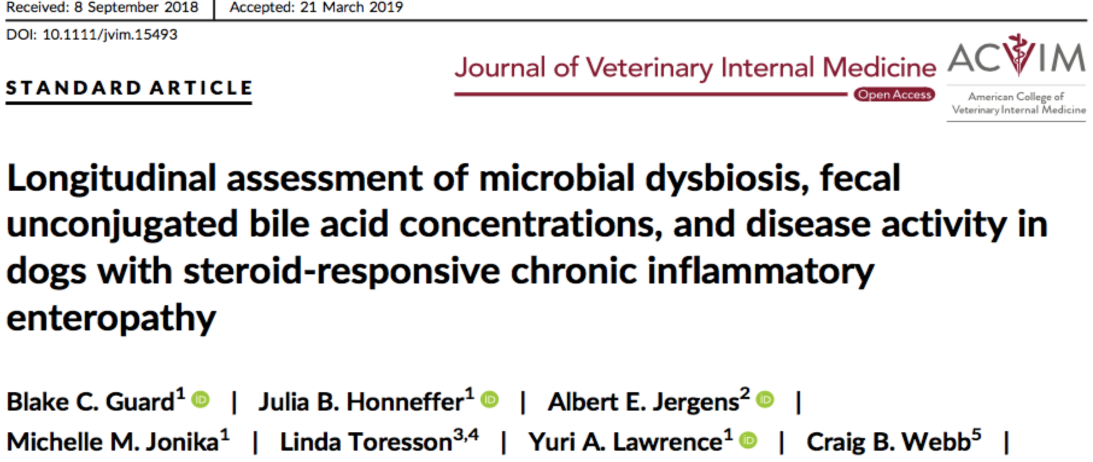
- Defective enterohepatic circulation

- Most commonly due to:
  - Malabsorption
  - Dysbiosis (lack of *P. hiranonis*)

⇛ ↑ ↑ total or 1 BAs in the colon

⇛ watery diarrhea, urgency, bloating, nightly diarrhea, soiling accidents, pain, attacks of abdominal cramping

Responds to bile acid sequestrants



Received: 8 September 2018 | Accepted: 21 March 2019

DOI: 10.1111/jvim.15493

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access ACVIM American College of Veterinary Internal Medicine

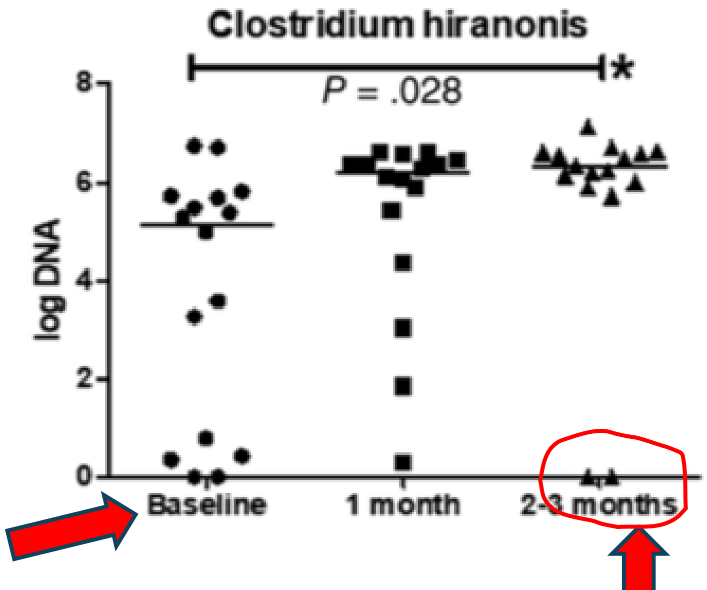
Longitudinal assessment of microbial dysbiosis, fecal unconjugated bile acid concentrations, and disease activity in dogs with steroid-responsive chronic inflammatory enteropathy

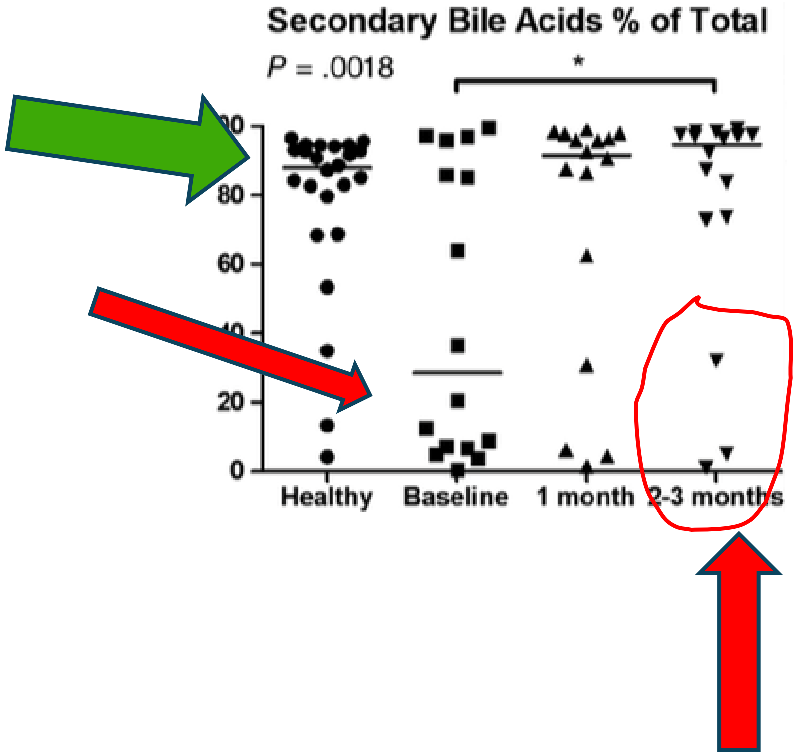
Blake C. Guard[1] | Julia B. Honneffer[1] | Albert E. Jergens[2] | Michelle M. Jonika[1] | Linda Toresson[3,4] | Yuri A. Lawrence[1] | Craig B. Webb[5] |

- Healthy dogs (n=24) significantly higher % of secondary BAs than dogs with CE (n=23)

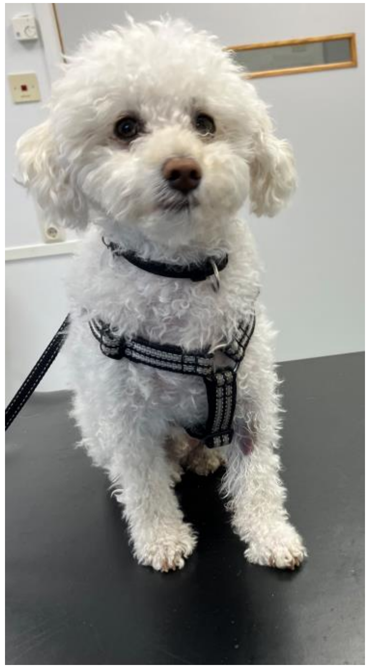
- % of secondary BAs improves in CE dogs with corticosteroid treatment

- Abundance of C hiranonis improves over time w corticosteroid treatment
- But not in some dogs.....





”Harry”, (♂) mixed breed, 4 Y/A



Referral, main complaint: nausea, severe borborygmia, signs of abdominal pain, lethargy.

Does not want to play or go for longer walks

Very difficult decrease corticosteroids; on daily tx

Tapering → worsening of borborygmia, lethargy and pain

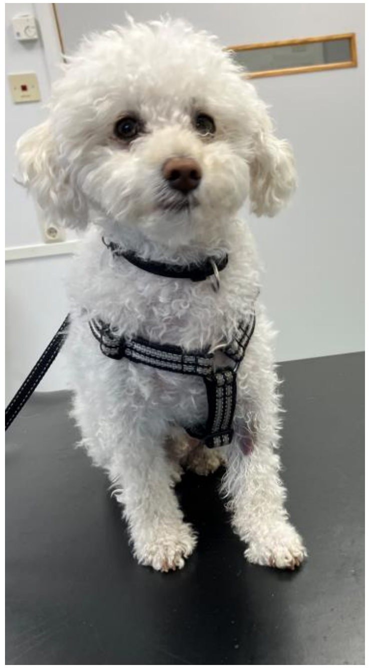
No diarrhea, vomiting before started on corticosteroids

Dietary trials: ”GI” diet, low fat diet, hydrolyzed soy based diet (current)

Corticosteroids q 24 hrs, CSA q 58 hrs

(1 mg budesonid q 48 hrs, 3.5 mg methylpred q 48 hrs)

”Harry”, (♂) mixed breed, 4 Y/A



Clin. ex: tense abdomen, vocalizes on abdominal palpation. Mild-mod. hypotrichosis, mild generalized erythema, mildly thin and paper-like skin

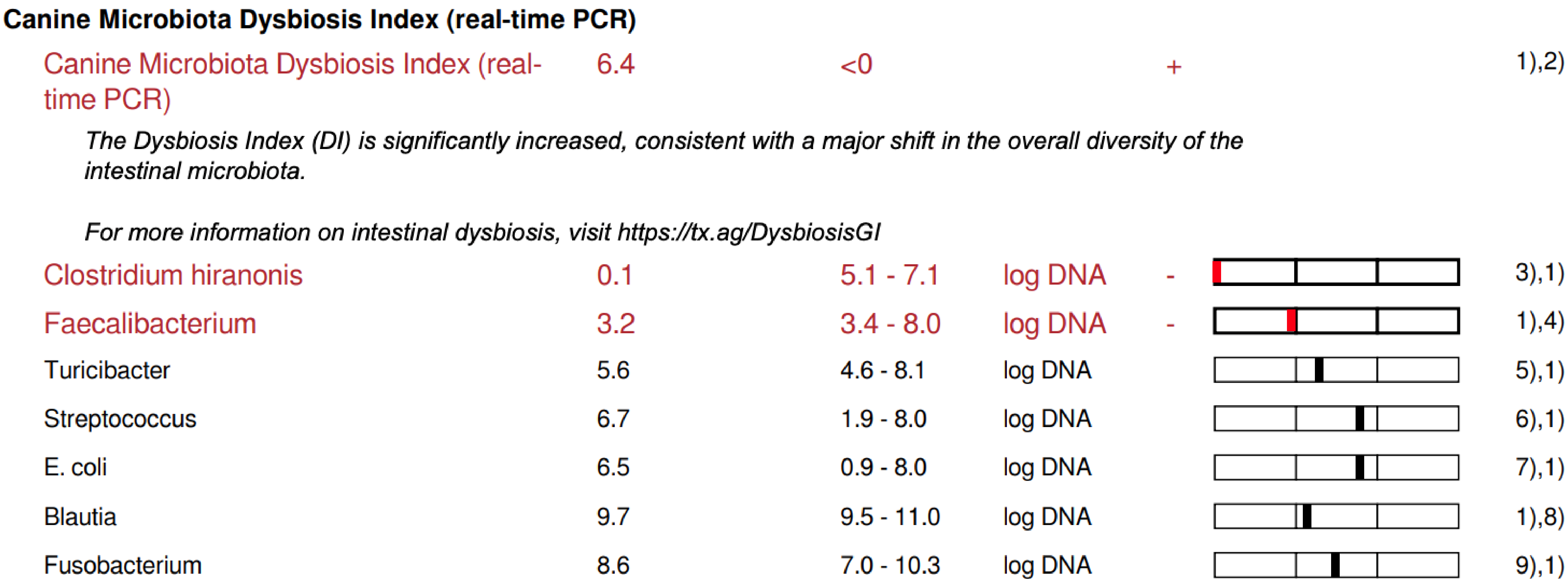
CBC + serum biochemistry: WNL

Abdominal ultrasound scan: mild amount of ”speckles” in the duodenum and jejunum, remaining WNL

Change of treatment: 1. New dietary trial 2. Stop CSA 3. +/- FMT

Home made balanced diet of horse meat and potatoe (w. nutritionist)

”Harry”, (♂) mixed breed, 4 Y/A



Canine Microbiota Dysbiosis Index (real-time PCR)

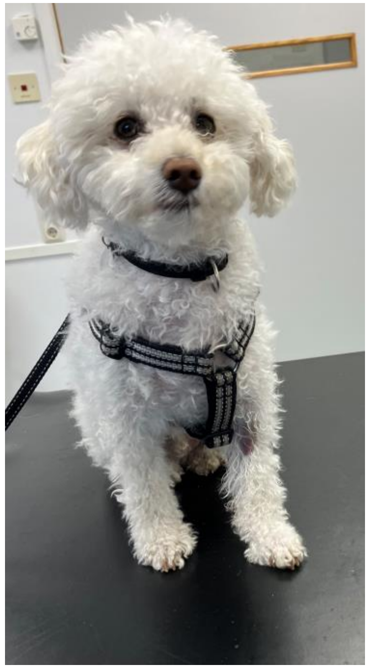
Canine Microbiota Dysbiosis Index (real-time PCR)	6.4	<0		+	1),2)

The Dysbiosis Index (DI) is significantly increased, consistent with a major shift in the overall diversity of the intestinal microbiota.

For more information on intestinal dysbiosis, visit https://tx.ag/DysbiosisGI

Clostridium hiranonis	0.1	5.1 - 7.1	log DNA	-	3),1)
Faecalibacterium	3.2	3.4 - 8.0	log DNA	-	1),4)
Turicibacter	5.6	4.6 - 8.1	log DNA		5),1)
Streptococcus	6.7	1.9 - 8.0	log DNA		6),1)
E. coli	6.5	0.9 - 8.0	log DNA		7),1)
Blautia	9.7	9.5 - 11.0	log DNA		1),8)
Fusobacterium	8.6	7.0 - 10.3	log DNA		9),1)

"Harry", (♂) mixed breed, 4 Y/A



1 month later: slightly more alert, some boborygmia but generally improved after diet change

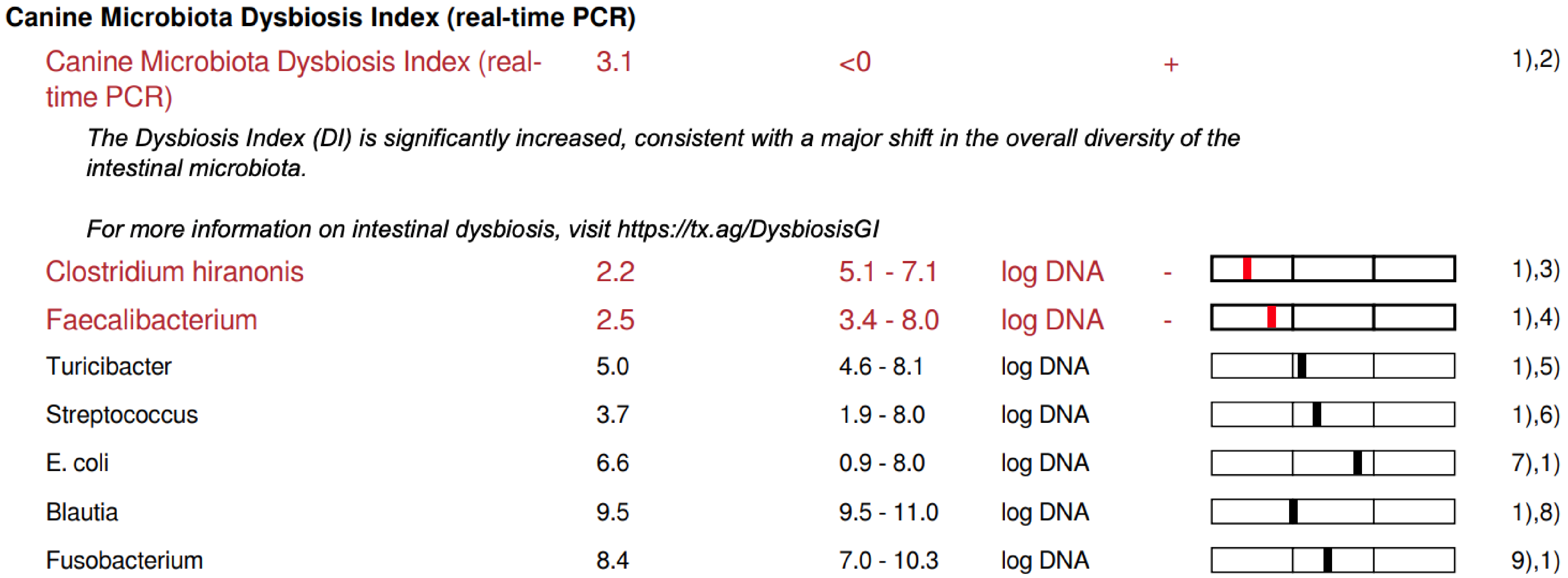
2 months later: More active, started playing a little

Tapered methylpred 0.5 mg q 48 hrs to 3 mg q 48 hrs – OK

→ **Further tapering not possible**

Still on 1 mg budesonide q 48 hrs

DI 2 months after new dietary trial



Canine Microbiota Dysbiosis Index (real-time PCR)

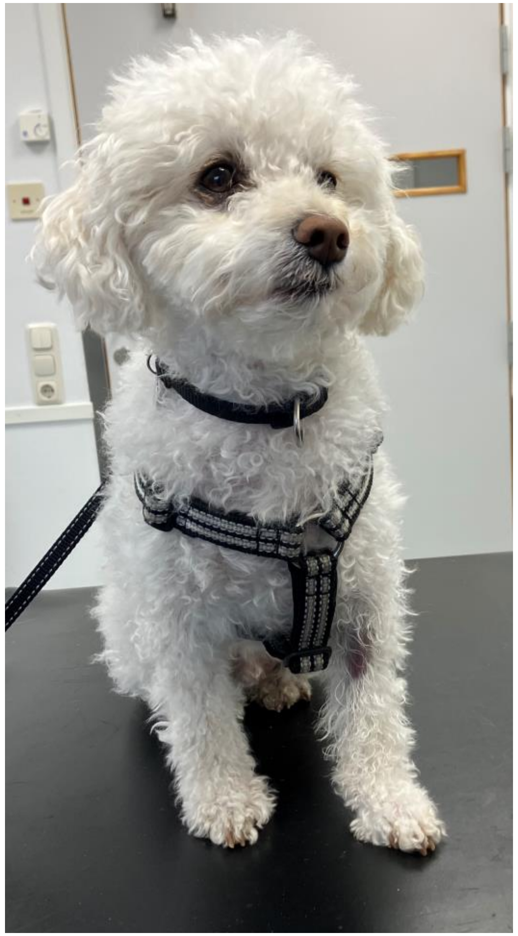
Canine Microbiota Dysbiosis Index (real-time PCR)	3.1	<0		+		1),2)

The Dysbiosis Index (DI) is significantly increased, consistent with a major shift in the overall diversity of the intestinal microbiota.

For more information on intestinal dysbiosis, visit https://tx.ag/DysbiosisGI

Clostridium hiranonis	2.2	5.1 - 7.1	log DNA	-		1),3)
Faecalibacterium	2.5	3.4 - 8.0	log DNA	-		1),4)
Turicibacter	5.0	4.6 - 8.1	log DNA			1),5)
Streptococcus	3.7	1.9 - 8.0	log DNA			1),6)
E. coli	6.6	0.9 - 8.0	log DNA			7),1)
Blautia	9.5	9.5 - 11.0	log DNA			1),8)
Fusobacterium	8.4	7.0 - 10.3	log DNA			9),1)

"Harry", (♂) mixed breed, 4 Y/A



Still requiring daily doses of corticosteroids

Marked dysbiosis and lack of *P. hiranonis* despite:

- 4 dietary trials
- 2 different immunomodulatory tx

Some people with BA dysmetabolism ⇒ abdominal pain, flatulence, lethargy, and bloating as main clinical sign

⇒ improves with BA sequestrants (Gupta et al 2015)

Started cholesevelam (BA sequestrants)

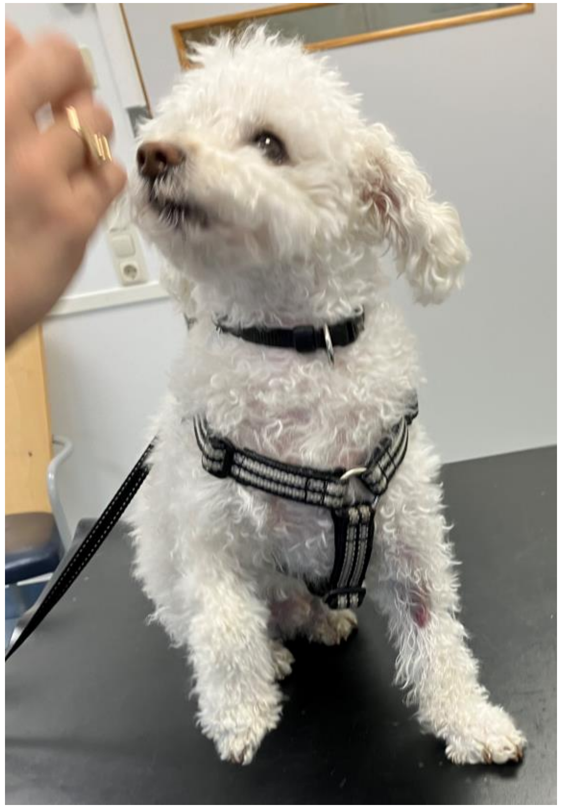
”Harry”, (♂) mixed breed, 4 Y/A

Within one week: much more active, started playing a lot in the evenings

Plays more than he has done in several years

No pain on abdominal palpation at two follow-up visits

Managed to slowly taper corticosteroids with 33%, continuing to slowly taper the dose



Clinical application of fecal DI

↑↑ **DI, especially with ↓↓ *P. hiranonis*** suggests presence of active CE

- useful as additional marker for chronic GI disease in animals with unspecific clinical signs
- useful as staging
- identify early changes in patients at risk – early intervention

- **Assess response to treatment**

- **Severely increased DI is associated with more severe changes in gut environment:**
  - may be a predictor for poorer long-term remission, as animals with persistent high DI have often relapse of clinical signs, and require long-term management
  - animals with high DI often have short-lived response to FMT, requiring repeated FMTs
  - persistently high DI with low P. hiranonis in dogs with clinical signs refractory to multi-modal therapy may be an indication for Bile Acid Malabsorption (BAM

- **Screen for healthy FMT donors and assess FMT outcome**

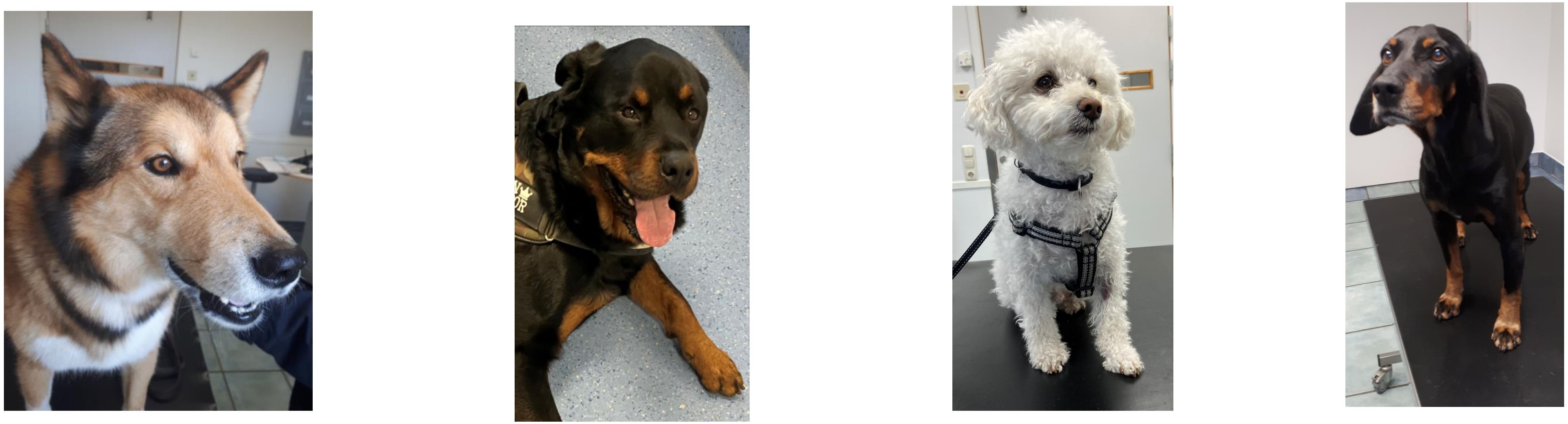
Toresson *et al. Canine Medicine and Genetics* (2021) 8:1
https://doi.org/10.1186/s40575-021-00099-x

Canine Medicine and Genetics

CASE REPORT Open Access

Cholestyramine treatment in two dogs with presumptive bile acid diarrhoea: a case report

L. Toresson[1,2*], J. M. Steiner[3] and J. S. Suchodolski[3]



TEXAS A&M UNIVERSITY
Gastrointestinal Laboratory

Thank you!
Questions?