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MICROBIOME FORUM

PUTTING MICROBIOME SCIENCE INTO CLINICAL PRACTICE

Purina Institute Microbiome Forum Virtual Event 2024

6–7 November 2024

Gut Villi

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Scientific Summaries

Microbiome research has generated a wealth of information, but the science can become lost in translation from research to actionable interventions in clinical veterinary practice. The Purina Institute Microbiome Forum Virtual Event is dedicated to providing clinically relevant microbiome science for practicing veterinarians.

The summaries presented here provide an overview of the scientific content as well as guidance for its translation to clinical practice. These summaries were prepared by the Purina Institute based on the content of the presentations and the Q&A and panel discussions and have been reviewed and approved by the presenters. Please note, summaries of the research reports have not been included because the studies have not yet been published. The summaries present the speakers' perspectives, and do not necessarily reflect the views of the Purina Institute or the Nestlé Purina PetCare Company or its affiliates.

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Incorporating the microbiome in daily clinical practice – case-based diagnostic and treatment approaches to chronic GI signs

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Chronic gastrointestinal (GI) disease has multifactorial pathophysiology involving the microbiota, epithelial barrier function, environmental triggers, and innate and adaptive immune system. Risk factors, especially early in life, include poor quality/imbalanced diet, enteropathogens/pathobionts, and antimicrobials. A thorough diagnostic workup is needed to fully evaluate and understand the patient's disease.

Chronic enteropathy (CE) is the most common cause of chronic recurrent or persistent GI signs and represents multilevel dysfunction and ultrastructural changes in the intestine, which may include mucosal proprial inflammatory infiltrates (lymphoplasmacytic, eosinophilic), epithelial injury, villous atrophy, fibrosis, lymphangiectasia, crypt disease, abnormal bacterial sensing and killing, and aberrant, excessive immune responses, and dysbiosis. Histopathologic evaluation of intestinal biopsies from dogs with CE generally show increased bacterial mucosal attachment, eosinophilic infiltration, and/or neutrophilic infiltration. However, histopathology overlaps across the CE phenotypes and does not predict the treatment response for the different categories of CE.

Traditionally, CE was classified based on sequential treatment trials: resolution or improvement in clinical signs in response to diet change translated to a diagnosis of food-responsive enteropathy (FRE), while those patients who did not meet the FRE diagnosis but responded to antibiotic administration were classified as antibiotic-responsive enteropathy (ARE) and those that failed to meet FRE or ARE categories but responded to immunosuppressant medications were diagnosed with immunosuppressant-responsive enteropathy (IRE). Patients that did not improve with any of these therapies were considered to have non-responsive enteropathy (NRE). However, this has evolved and the existence of ARE has been increasingly challenged. In addition, the use of antibiotics is discouraged for the majority of CE cases due to the risk of relapse, the risk of antimicrobial resistance and its risks to public health, and their induction of long-lasting dysbiosis. In reality, the percentage of CE cases that are actually FRE is much higher than most veterinarians realize.

Dysbiosis, an imbalance of the gut microbiome, can result from reduction in commensal organisms or an increase in pathobionts and can induce clinical signs through a number of pathological mechanisms:

- Inflammation due to increased bacterial numbers and metabolic products
- Inflammation in response to increases of mucosa-adhering bacteria
- Osmotic diarrhea resulting from the accumulation of excess and/or abnormal substrates in the intestinal lumen and increased bacterial fermentation
- Overgrowth of pathobionts and reduction of beneficial bacteria, resulting in inflammation and reduced bile acid conversion

  The Dysbiosis Index (DI) uses rapid quantitative PCR to determine the absolute abundance of total bacteria and 7 key bacterial groups (5 of which are beneficial, and 2 are pathobionts) to reflect shifts in the gut microbiota. Some of these bacteria are increased in dysbiosis, while others are decreased. The beneficial bacteria Peptaceobacter hiranonis (formerly Clostridium hiranonis) is the primary organism responsible for bile acid conversion in dogs and cats and its abundance is a key player in dysbiosis; 50-70% of dogs and 30% of cats with CE have decreased P. hiranonis on the DI.

A DI above 2 in dogs (1 in cats) indicates significant dysbiosis and a DI of 0-2 in dogs (0-1 in cats) indicates minor dysbiosis. CE cases commonly have dysbiosis. Acute enteropathy may cause a slight shift to dysbiosis, but it resolves quickly. Antimicrobials induce dysbiosis which can be severe and persist weeks to months after the cessation of the antibiotic.

In dogs, CE is not always associated with dysbiosis. Dogs with CE but without dysbiosis tend to have microbial populations that more closely resemble those of healthy dogs, whereas dogs with CE and dysbiosis are noticeably separate; the question is whether this represents two distinct manifestations of CE or if dogs with clinical signs of CE but without dysbiosis actually have CE. The DI can also be a sensitive indicator of a change in the intestinal environment. In cats, the DI becomes abnormal before other markers of intestinal disease. The DI also serves as a prognostic marker for the likelihood of achieving clinical control; the likelihood is better with eubiosis (normal DI value) or mild dysbiosis compared to more severe dysbiosis.

The presence and severity of dysbiosis may help us stage CE cases. With a multifactorial pathophysiology, various overlapping pathologies exist in CE which differ in extent between patients. One subset of patients has increased DI, increased markers of inflammation, malabsorption, and abnormal intestinal permeability. CE may be characterized by persistent dysfunction, persistent dysbiosis, and metabolomic changes. When the DI indicates marked dysbiosis, the pathology is usually significant and case management should be focused on clinical remission for as long as possible.

Staging of intestinal changes associated with CE will allow better understanding of underlying pathologies and prognosis, and guide treatment choices (multimodal vs sequential) for individual cases. Staging may be based on the DI, signs of malabsorption (e.g., hypocobalaminemia, fecal BA dysmetabolism), mucosal remodeling, and mucosal inflammation.

Fecal microbiota transplantation (FMT) is a microbiome-centric approach with growing promise for management of CE cases. Recent studies have shown a clinical response to 1-3 FMTs in 72-100% of cases, with improved CIBDAI and fecal scores in dogs with CE that had not responded to diet (Toresson et al, 2022; Vecchiato et al, 2023) FMT can help restore P. hiranonis. Multiple FMTs may be necessary to achieve clinical improvement in some dogs with CE, and the presence of severe dysbiosis with or without reduced P. hiranonis increases the likelihood that subsequent FMTs will be needed.

Although there is limited data on the long-term safety of FMT, clinical experience to date indicates it is very safe. Short-term adverse reactions are uncommon, but mirror the clinical signs associated with the pet's original presentation. Caution is advised when considering FMT in severely immunocompromised/predisposed patients. The procedure is very simple and can be performed in general practice.

Putting the science into practice:

- Chronic enteropathy (CE) has multifactorial pathophysiology involving the microbiome, intestinal tract, immune system, and environment.
- The percentage of food-responsive CE is much higher than most veterinarians realize.
- Do not classify the CE or predict its treatment response based on histopathology.
- Dysbiosis is an imbalance of the gut microbiome and the presence of moderate or severe dysbiosis with CE is often indicative of more severe pathology.
- The dysbiosis index (DI) is a valuable clinical tool that can identify early changes in at-risk patients, facilitating early intervention; increase suspicion of CE in patient with recurrent/acute-on-chronic GI signs; assess response to treatment; predict the likelihood of long-term clinical response to FMT; and screen healthy pets for their suitability as FMT donors.
- A multimodal approach to CE should begin with diet but may also include FMT, probiotics, and immunosuppressive drugs.
    - The use of antibiotics is discouraged for the majority of CE cases due to the risk of relapse, the risk of antimicrobial resistance and its risks to public health, and their induction of long-lasting dysbiosis

References:

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

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(11), 589–599. doi: 10.1111/jsap.12588

Doulidis, P. G., Galler, A. I., Hausmann, B., Berry, D., Rodríguez-Rojas, A., & Burgener, I. A. (2023). Gut microbiome signatures of Yorkshire Terrier enteropathy during disease and remission. Scientific Reports, 13(1), 4337. doi: 10.1038/s41598-023-31024-w

Dupouy-Manescau, N., Méric, T., Sénécat, O., Drut, A., Valentin, S., Leal, R. O., & Hernandez, J. (2024). Updating the classification of chronic inflammatory enteropathies in dogs. Animals, 14(5), 681. doi: 10.3390/ani14050681

Jergens, A. E., & Heilmann, R. M. (2022). Canine chronic enteropathy—Current state-of-the-art and emerging concepts. Frontiers in Veterinary Science, 9, 923013. doi: 10.3389/fvets.2022.923013

Kathrani, A., Blackwell, E. J., Williams, J. L., Gruffydd-Jones, T., Murray, J. K., Hezzell, M., & Hall, E. J. (2019). Exploring early life events including diet in cats presenting for gastrointestinal signs in later life. Veterinary Record, 185(5), 144–144. doi: 10.1136/vr.105040

Sato-Takada, K., Flemming, A. M., Voordouw, M. J., & Carr, A. P. (2022). Parvovirus enteritis and other risk factors associated with persistent gastrointestinal signs in dogs later in life: A retrospective cohort study. BMC Veterinary Research, 18(1), 96. doi: 10.1186/s12917-022-03187-7

Skotnitzki, E., Suchodolski, J. S., Busch, K., Werner, M., Zablotski, Y., Ballhausen, B. D., Neuerer, F., & Unterer, S. (2022). Frequency of signs of chronic gastrointestinal disease in dogs after an episode of acute hemorrhagic diarrhea. Journal of Veterinary Internal Medicine, 36(1), 59–65. doi: 10.1111/jvim.16312

Stavroulaki, E. M., Suchodolski, J. S., Pilla, R., Fosgate, G. T., Sung, C.-H., Lidbury, J. A., Steiner, J. M., & Xenoulis, P. G. (2021). Short- and long-term effects of amoxicillin/clavulanic acid or doxycycline on the gastrointestinal microbiome of growing cats. PLOS ONE, 16(12), e0253031. doi: 10.1371/journal.pone.0253031

Suchodolski, J. (2023). Assessing and managing the gut microbiome in canine and feline practice. In Purina Institute Handbook of Canine and Feline Clinical Nutrition (2nd ed., pp. 113–118). Embark Consulting Group, LLC.

Sung, C.-H., Marsilio, S., Chow, B., Zornow, K. A., Slovak, J. E., Pilla, R., Lidbury, J. A., Steiner, J. M., Park, S. Y., Hong, M.-P., Hill, S. L., & Suchodolski, J. S. (2022). Dysbiosis index to evaluate the fecal microbiota in healthy cats and cats with chronic enteropathies. Journal of Feline Medicine and Surgery, 24(6), e1–e12. doi: 10.1177/1098612X221077876

Sung, C.-H., Pilla, R., Chen, C.-C., Ishii, P. E., Toresson, L., Allenspach-Jorn, K., Jergens, A. E., Summers, S., Swanson, K. S., Volk, H., Schmidt, T., Stuebing, H., Rieder, J., Busch, K., Werner, M., Lisjak, A., Gaschen, F. P., Belchik, S. E., Tolbert, M. K., … Suchodolski, J. S. (2023). Correlation between targeted qPCR assays and untargeted DNA shotgun metagenomic sequencing for assessing the fecal microbiota in dogs. Animals, 13(16), 2597. doi: 10.3390/ani13162597

Toresson, L., Spillmann, T., Pilla, R., Ludvigsson, U., Hellgren, J., Olmedal, G., & Suchodolski, J. S. (2023). Clinical effects of faecal microbiota transplantation as adjunctive therapy in dogs with chronic enteropathies—A retrospective case series of 41 dogs. Veterinary Sciences, 10(4), 271. doi: 10.3390/vetsci10040271

Torres, J., Burisch, J., Riddle, M., Dubinsky, M., & Colombel, J.-F. (2016). Preclinical disease and preventive strategies in IBD: Perspectives, challenges and opportunities. Gut, 65(7), 1061–1069. doi: 10.1136/gutjnl-2016-311785

Vecchiato, C., Erba, D., Sportelli, F., Delsante, C., Alonzo, M., Pinna, C., Sabetti, M., Suchodolski, J., Pilla, R., Giaretta, P., Pietra, M., Biagi, G., & Procoli, F. (2023). Fecal microbial transplantation effect on clinical outcome and fecal microbiota and metabolome in dogs with chronic enteropathy refractory to diet. Research Communications of the 33rd ECVIM-CA Congress, 743. doi: 10.1111/jvim.16930

Volkmann, M., Steiner, J. M., Fosgate, G. T., Zentek, J., Hartmann, S., & Kohn, B. (2017). Chronic diarrhea in dogs – Retrospective study in 136 cases. Journal of Veterinary Internal Medicine, 31(4), 1043–1055. doi: 10.1111/jvim.14739

Wang, S., Martins, R., Sullivan, M. C., Friedman, E. S., Misic, A. M., El-Fahmawi, A., De Martinis, E. C. P., O'Brien, K., Chen, Y., Bradley, C., Zhang, G., Berry, A. S. F., Hunter, C. A., Baldassano, R. N., Rondeau, M. P., & Beiting, D. P. (2019). Diet-induced remission in chronic enteropathy is associated with altered microbial community structure and synthesis of secondary bile acids. Microbiome, 7(1), 126. doi: 10.1186/s40168-019-0740-4

Winston, J. A., Suchodolski, J. S., Gaschen, F., Busch, K., Marsilio, S., Costa, M. C., Chaitman, J., Coffey, E. L., Dandrieux, J. R. S., Gal, A., Hill, T., Pilla, R., Procoli, F., Schmitz, S. S., Tolbert, M. K., Toresson, L., Unterer, S., Valverde-Altamirano, É., Verocai, G. G., … Ziese, A.-L. (2024). Clinical guidelines for fecal microbiota transplantation in companion animals. Advances in Small Animal Care, 5(1), 79–107. doi: 10.1016/j.yasa.2024.06.006

Resident *E. coli*: a frenemy within

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Escherichia coli is an early colonizer in humans, puppies, and kittens, and has variable virulence. Its role in the microbiome exists on a spectrum; it can be a symbiont, opportunistic pathogen, or pathogen (with variable pathogenicity).

E. coli is genetically diverse, with only 40% of its pangenome considered core, and is highly adapted to life at the intestinal mucosal interface. Its colonization density and spatial distribution differ in health and disease; in health, the mucus layer is its "happy place," whereas disease is associated with epithelial contact, mucosal invasion and translocation. Increased relative and absolute abundance of *E. coli* and mucosal association are observed in dogs and cats with chronic enteropathy (CE), people with ileal Crohn's disease and French Bulldogs and Boxers with PAS+ granulomatous colitis. Remission of PAS+ granulomatous colitis in Boxers and French bulldogs correlates with eradication of invasive intramucosal *E. coli,* with sustained remission contingent on eradication and restoration of the mucosal barrier.

Inflammation may drive *E. coli*-associated dysbiosis, and vice versa, particularly in a genetically predisposed host; this can create and propagate a self-perpetuating cycle of worsening dysbiosis and inflammation. The number of *E. coli* and the degree of inflammation are associated with more severe disease. Inflammation may also act as a trigger for *E. coli* proliferation ("dysbiotic bloom"), virulence and translocation. *E. coli* is very adaptable when it comes to metabolism, and thrives in hostile microaerophilic gut environments. Inflammation associated mucosal metabolites fuel the growth and virulence of *E. coli* while suppressing commensals that are more susceptible to microenvironmental and nutrient stress. A gradient of inflammation associated amino acids may serve to chemoattract adherent invasive *E. coli* (AIEC) to the epithelial layer, while facilitating co-metabolism of other nutrients, e.g. ethanolamine. Metagenomic analysis of feces from dogs with diet responsive chronic enteropathy prior to diet induced remission reveals enhanced bacterial non-glucose carbon use, gluconeogenesis, beta-oxidation, glyoxalate shunt, anaerobic metabolism, and resistance to oxidative stress, with suppressed amino acid metabolism, B vitamin synthesis, and urea cycle.

What other factors, apart from inflammation, can drive *E. coli*-associated dysbiosis? Bones and raw food (BARF) diets are associated with higher levels of *E. coli*, including *E. coli* with diarrheagenic virulence genes. Antibiotics may drive dysbiosis through selective targeting of susceptible species, and altered synthesis of metabolites that modulate the microbiome e.g. secondary bile acids. Metronidazole administration induces a bloom of proteobacteria, especially *E. coli*.

The presence or absence of *E. coli* on fecal culture is not helpful in the diagnosis of *E. coli*-associated dysbiosis. Management of *E. coli*-associated dysbiosis should be based on integrated analysis of clinical findings, histopathology (or expected histopathology e.g. lymphoplasmacytic enteritis vs. granulomatous colitis), and *E. coli* colonization density and spatial distribution. Is *E. coli* confined to lumen, attached to the epithelium, invading the mucosa, persisting in macrophages, or translocating? This can be evaluated with Fluorescence in Situ Hybridization (FISH) of intestinal biopsies procured for histopathology. Antibiotics are reserved for patients with evidence of bacterial invasion, translocation or persistent infections in gut mucosa or other sites. In cats with neutrophilic inflammatory liver disease/cholangitis/cholecystitis and /or moderate to severe pancreatitis and/ or IBD/lymphoma, "triaditis", translocation of enteric *E. coli*, *Enterococcus* and *Streptococcus* spp. has been documented by microbial culture and FISH.

In dogs with food -responsive chronic enteropathy, diets that induce clinical remission (hydrolyzed and ingredient restricted) also alleviated *E. coli* and *C. perfringens* dysbiosis. This may be related to restoration of bile acid metabolism, with concurrent reduction of primary bile

acids that can stimulate growth of *E. coli* and an increase in secondary bile acids that can suppress *E. coli* (and *C. perfringens*). Diet-induced clinical remission also alleviates inflammation-associated metabolic stress, with inflammation, dysbiosis, and metabolic perturbations shifting closer to health.

In addition to diet, future strategies to combat *E. coli*-associated dysbiosis include pre-, pro- and post-biotics, fecal microbiota transplantation (FMT), and selective antagonism of *E. coli* growth and virulence. Supplemental prebiotics that support the growth of commensal probiotic bacteria and/or probiotic bacteria may restore the microbial and chemical microenvironment to selectively out-compete *E. coli* for niche and nutrients, constraining dysbiosis. Postbiotics, such as those derived from *F. prausnitzii,* may reduce *E. coli* growth and virulence. FMT may restore the global microbial and chemical microenvironment. Ongoing research is evaluating the use of bacteriophages and substances to selectively block *E. coli* adhesion/invasion and inhibit metabolism, growth, motility, and virulence gene expression.

Putting the science into practice:

- *E. coli* can be a symbiont, opportunistic pathogen, or pathogen. The presence or absence of *E. coli* on fecal culture is not helpful in the diagnosis of *E. coli*-associated dysbiosis.
- Inflammation, antibiotics and raw meat diets can create an environment that favors *E. coli* survival and growth, and the resulting dysbiosis may increase inflammation – creating a vicious self-fueling cycle, especially in genetically predisposed hosts.
- In health, *E. coli* is found in the lumen and mucus. In disease, mucosal association is increased, with adherent and invasive *E. coli* organisms linked to disease and associated pathology.
- Management of *E. coli*-associated dysbiosis should be based on integrated analysis of clinical findings, histopathology (or likely histopathology), and the number and spatial distribution of *E. coli* .
- In dogs with food responsive enteropathy the primary approach to managing *E. coli*-associated dysbiosis is feeding a high quality ingredient-restricted diet.

References:

Baumgart, M., Dogan, B., Rishniw, M., Weitzman, G., Bosworth, B., Yantiss, R., Orsi, R. H., Wiedmann, M., McDonough, P., Kim, S. G., Berg, D., Schukken, Y., Scherl, E., & Simpson, K. W. (2007). Culture independent analysis of ileal mucosa reveals a selective increase in invasive *Escherichia coli* of novel phylogeny relative to depletion of Clostridiales in Crohn's disease involving the ileum. *The ISME Journal*, *1*(5), 403–418. doi: 10.1038/ismej.2007.52

Chevalier, G., Laveissière, A., Desachy, G., Barnich, N., Sivignon, A., Maresca, M., Nicoletti, C., Di Pasquale, E., Martinez-Medina, M., Simpson, K. W., Yajnik, V., Sokol, H., MOBIDIC Study Investigators, Adegbamigbe, T., Ahmad, T., Arnott, I., Bouhnik, Y., Carbonnel, F., Colombel, J.-F., … Bonny, C. (2021). Blockage of bacterial FimH prevents mucosal inflammation associated with Crohn's disease. *Microbiome*, *9*(1), 176. doi: 10.1186/s40168-021-01135-5

Craven, M., Egan, C. E., Dowd, S. E., McDonough, S. P., Dogan, B., Denkers, E. Y., Bowman, D., Scherl, E. J., & Simpson, K. W. (2012). Inflammation drives dysbiosis and bacterial invasion in murine models of ileal Crohn's disease. *PLoS ONE*, *7*(7), e41594. doi: 10.1371/journal.pone.0041594

Del Carro, A., Corrò, M., Bertero, A., Colitti, B., Banchi, P., Bertolotti, L., & Rota, A. (2022). The evolution of dam-litter microbial flora from birth to 60 days of age. *BMC Veterinary Research*, *18*(1), 95. doi: 10.1186/s12917-022-03199-3

Dogan, B., Zhang, S., Kalla, S. E., Dogan, E. I., Guo, C., Ang, C. R., & Simpson, K. W. (2020). Molecular and phenotypic characterization of *Escherichia coli* associated with granulomatous colitis of Boxer dogs. *Antibiotics*, *9*(9), 540. doi: 10.3390/antibiotics9090540

Ghosh, T. S., Shanahan, F., & O'Toole, P. W. (2022). The gut microbiome as a modulator of healthy ageing. *Nature Reviews Gastroenterology & Hepatology*, *19*(9), 565–584. doi: 10.1038/s41575-022-00605-x

Gibson, J. F., Parker, V. J., Howard, J. P., Snell, C. M., Cross, E. W., Pagliughi, L. B., Diaz-Campos, D., Winston, J. A., & Rudinsky, A. J. (2022). *Escherichia coli* pathotype contamination in raw canine diets. *American Journal of Veterinary Research*, *83*(6), ajvr.21.10.0166. doi: 10.2460/ajvr.21.10.0166

Gookin, J. L., Strong, S. J., Bruno-Bárcena, J. M., Stauffer, S. H., Williams, S., Wassack, E., Azcarate-Peril, M. A., Estrada, M., Seguin, A., Balzer, J., & Davidson, G. (2022). Randomized placebo-controlled trial of feline-origin Enterococcus hirae probiotic effects on preventative health and fecal microbiota composition of fostered shelter kittens. *Frontiers in Veterinary Science*, *9*, 923792. doi: 10.3389/fvets.2022.923792

Gueta, R., Hader, C., Mueller, A. S., Heusinger, T., & Mueller E. (2019). Prevalence of diarrheagenic *Escherichia coli* bacteria in fecal samples from dogs showing signs of gastrointestinal disorders and a clinically healthy control group. *JSMC Microbiololgy*, *1*, 5.

Herlekar D, Martin FP, Zhang S, Bosworth B, Monyloliu I, Collino S, Yantiss, R, Kochnar S, Scherl EJ, Dogan B, Simpson KW. Chemical Perturbations in the inflamed ileum promotes the groath of Crohn's associated Adherent and Invasive E.coli. *Inflammatory Bowel Diseases*, Volume 19, Issue suppl_1, 1 December 2013, Page S121,P-239, https://doi.org/10.1097/01.MIB.0000439037.03219.2b

Herlekar D, Stewart K, Dogan B, Altier C, Scherl EJ, Simpson KW. Short Chain Fatty Acids and Bile Salts Modulate the Growth of Crohn's-Associated Adherent and InvasiveE. coli(AIEC). Gastroenterology 142(5):S-680. M01766. DOI: 10.1016/S0016-5085(12)62620-1

Janeczko, S., Atwater, D., Bogel, E., Greiter-Wilke, A., Gerold, A., Baumgart, M., Bender, H., McDonough, P. L., McDonough, S. P., Goldstein, R. E., & Simpson, K. W. (2008). The relationship of mucosal bacteria to duodenal histopathology, cytokine mRNA, and clinical disease activity in cats with inflammatory bowel disease. *Veterinary Microbiology*, *128*(1–2), 178–193. doi: 10.1016/j.vetmic.2007.10.014

Kittana, H., Gomes-Neto, J. C., Heck, K., Juritsch, A. F., Sughroue, J., Xian, Y., Mantz, S., Segura Muñoz, R. R., Cody, L. A., Schmaltz, R. J., Anderson, C. L., Moxley, R. A., Hostetter, J. M., Fernando, S. C., Clarke, J., Kachman, S. D., Cressler, C. E., Benson, A. K., Walter, J., & Ramer-Tait, A. E. (2023). Evidence for a causal role for *Escherichia coli* strains identified as adherent-invasive (AIEC) in intestinal inflammation. *mSphere*, *8*(2), e00478-22. doi: 10.1128/msphere.00478-22

Li, H., Limenitakis, J. P., Fuhrer, T., Geuking, M. B., Lawson, M. A., Wyss, M., Brugiroux, S., Keller, I., Macpherson, J. A., Rupp, S., Stolp, B., Stein, J. V., Stecher, B., Sauer, U., McCoy, K. D., & Macpherson, A. J. (2015). The outer mucus layer hosts a distinct intestinal microbial niche. *Nature Communications*, *6*(1), 8292. doi: 10.1038/ncomms9292

Manchester, A. C., Dogan, B., Guo, Y., & Simpson, K. W. (2021). *Escherichia coli*-associated granulomatous colitis in dogs treated according to antimicrobial susceptibility profiling. *Journal of Veterinary Internal Medicine*, *35*(1), 150–161. doi: 10.1111/jvim.15995

Morgan, X. C., Miller, M. L., Loftus, J. P., Frederick, C. E., Wakshlag, J. J., Zhang, S., Rishniw, M., & Simpson, K. W. (2024, September 5-7). Limited ingredient diets remodel the microbiome and metabolome of dogs with food-responsive enteropathy. [Conference presentation]. ECVIM 2024, Lyon, France.

Osbaldiston, G. W., & Stowe, E. C. (1971). Microflora of alimentary tract of cats. *American Journal of Veterinary Research, 32*(9), 1399-1405.

Pilla, R., Gaschen, F. P., Barr, J. W., Olson, E., Honneffer, J., Guard, B. C., Blake, A. B., Villanueva, D., Khattab, M. R., AlShawaqfeh, M. K., Lidbury, J. A., Steiner, J. M., & Suchodolski, J. S. (2020). Effects of metronidazole on the fecal microbiome and metabolome in healthy dogs. *Journal of Veterinary Internal Medicine*, *34*(5), 1853–1866. doi: 10.1111/jvim.15871

Puño-Sarmiento, J., Medeiros, L., Chiconi, C., Martins, F., Pelayo, J., Rocha, S., Blanco, J., Blanco, M., Zanutto, M., Kobayashi, R., & Nakazato, G. (2013). Detection of diarrheagenic Escherichia coli strains isolated from dogs and cats in Brazil. *Veterinary Microbiology*, *166*(3–4), 676–680. doi: 10.1016/j.vetmic.2013.07.007

Safety and efficacy of EcoActive on intestinal adherent invasive *E. Coli* in patients with inactive Crohn's disease ClinicalTrials.gov ID NCT03808103

Schmidt, V. M., Pinchbeck, G., McIntyre, K. M., Nuttall, T., McEwan, N., Dawson, S., & Williams, N. J. (2018). Routine antibiotic therapy in dogs increases the detection of antimicrobial-resistant faecal *Escherichia coli*. *Journal of Antimicrobial Chemotherapy*. doi: 10.1093/jac/dky352

Secher, T., Brehin, C., & Oswald, E. (2016). Early settlers: Which *E. coli* strains do you not want at birth? *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *311*(1), G123–G129. doi: 10.1152/ajpgi.00091.2016

Simpson, K. W., Dogan, B., Rishniw, M., Goldstein, R. E., Klaessig, S., McDonough, P. L., German, A. J., Yates, R. M., Russell, D. G., Johnson, S. E., Berg, D. E., Harel, J., Bruant, G., McDonough, S. P., & Schukken, Y. H. (2006). Adherent and Invasive *Escherichia coli* Is associated with granulomatous colitis in Boxer dogs. *Infection and Immunity*, *74*(8), 4778–4792. doi: 10.1128/IAI.00067-06

Simpson, K. W., Miller, M. L., Loftus, J. P., Rishniw, M., Frederick, C. E., & Wakshlag, J. J. (2023). Randomized controlled trial of hydrolyzed fish diets in dogs with chronic enteropathy. *Journal of Veterinary Internal Medicine*, *37*(6), 2334–2343. doi: 10.1111/jvim.16844.

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*(3–4), 394–400. doi: 10.1016/j.vetmic.2009.11.002

U.S. Food and Drug Administration (2024) Outbreak investigation of E. coli 0157:H7: McDonalds Quarter Pounders (October 2024)

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

Wang, S., Martins, R., Sullivan, M. C., Friedman, E. S., Misic, A. M., El-Fahmawi, A., De Martinis, E. C. P., O'Brien, K., Chen, Y., Bradley, C., Zhang, G., Berry, A. S. F., Hunter, C. A., Baldassano, R. N., Rondeau, M. P., & Beiting, D. P. (2019). Diet-induced remission in chronic enteropathy is associated with altered microbial community structure and synthesis of secondary bile acids. *Microbiome*, *7*(1), 126. doi: 10.1186/s40168-019-0740-4

Watson, V. E., Jacob, M. E., Flowers, J. R., Strong, S. J., DebRoy, C., & Gookin, J. L. (2017). Association of atypical enteropathogenic *Escherichia coli* with diarrhea and related mortality in kittens. *Journal of Clinical Microbiology*, *55*(9), 2719–2735. doi: 10.1128/JCM.00403-17

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: Small intestinal microbial communities in canine IBD. *FEMS Microbiology Ecology*, *66*(3), 579–589. doi: 10.1111/j.1574-6941.2008.00556.x

Young, W., Moon, C. D., Thomas, D. G., Cave, N. J., & Bermingham, E. N. (2016). Pre- and post-weaning diet alters the faecal metagenome in the cat with differences in vitamin and carbohydrate metabolism gene abundances. *Scientific Reports*, *6*(1), 34668. doi: 10.1038/srep34668

Zhang, S., Dogan, B., Guo, C., Herlekar, D., Stewart, K., Scherl, E. J., & Simpson, K. W. (2020). Short chain fatty acids modulate the growth and virulence of pathosymbiont *Escherichia coli* and host response. *Antibiotics*, *9*(8), 462. doi: 10.3390/antibiotics9080462.

Zhang, S., Fu, J., Dogan, B., Scherl, E. J., & Simpson, K. W. (2018). 5-Aminosalicylic acid downregulates the growth and virulence of Escherichia coli associated with IBD and colorectal cancer, and upregulates host anti-inflammatory activity. *The Journal of Antibiotics*, *71*(11), 950–961. doi: 10.1038/s41429-018-0081-8

Zhang, S., Morgan, X., Dogan, B., Martin, F.-P., Strickler, S., Oka, A., Herzog, J., Liu, B., Dowd, S. E., Huttenhower, C., Pichaud, M., Dogan, E. I., Satsangi, J., Longman, R., Yantiss, R., Mueller, L. A., Scherl, E. J., Sartor, R. B., & Simpson, K. W. (2022). Mucosal metabolites fuel the growth and virulence of E. coli linked to Crohn's disease. *JCI Insight*, *7*(10), e157013. doi: 10.1172/jci.insight.157013

Practical management of food-responsive enteropathies – how to perform proper dietary trials

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Performing a proper dietary trial is critical to the diagnosis and management of numerous gastrointestinal (GI) conditions, and success is determined by proper diet selection, owner and pet compliance with the diet, and monitoring the response to determine efficacy and next steps.

Selecting the most appropriate diet

Signalment, appetite, and body weight/condition are important considerations, as well as documentation of GI signs such as vomiting/regurgitation/nausea, diarrhea (small bowel, large bowel, mixed), and weight loss/stability when selecting the most appropriate diet.

A **diet history** is critical because it allows veterinarians to determine the pet's and owner's general preferences for texture, flavors, and wet vs. dry food. It also allows an evaluation of how the diet composition (e.g., ingredients, fat, fiber) correlates with gastrointestinal (GI) signs – have the owners recognized a specific diet that has helped the most? This may help drive diet selection for the food trial. The diet history can also identify which therapeutic diets have been tried, for how long they were fed, and how well the owners complied with feeding instructions. The owners may claim to have tried a therapeutic diet but if they did not feed the diet exclusively, or for long enough (at least 2 weeks) that diet should not be ruled out as a possible diet for the trial.

Below is a short overview of the advantages and disadvantages of diet category options for elimination trials:

Diet type	Pros	Cons
GI diet	Highly digestible May be less expensive Increased palatability Some formulas have low fat	Ability to maintain remission long term may be reduced
Novel protein	Increased palatability Scientific studies show remission rate of 50-60% in dogs and cats with chronic enteropathy	Adequate diet history needed to determine what will truly be novel for that patient (and it may be difficult to find an available novel protein) Fat content (low fat not as available), Anecdotally some tend to relapse after a period of feeding
Commercial therapeutic hydrolyzed	No diet history needed Anecdotally less chance of relapse Anecdotal & scientific studies support a two-third response rate in dogs and cats with chronic enteropathy Controlled and low-fat formulas available	Reduced palatability (20-25% of canine cases don't transition well; success rate actually higher in cats than dogs) Limited canned formulas for cats May be expensive compared to some other categories

Commercial therapeutic fiber-enriched diet	Multiple studies support use for chronic colitis and some enterocolitis cases Safe & well-tolerated Response relatively quick (mean 8.5 days in one study, 1 day in another)	Long-term supplementation may be required Reduced energy density, may be a concern in inappetent, underconditioned animals Delay gastric emptying depending on fiber type (may exacerbate regurgitation, vomiting, nausea)
Home-prepared	Increased digestibility Increased palatability Precisely set macronutrient profile to account for comorbidities, etc. Avoids antigens from processing (but not a common need)	Access to board-certified veterinary nutritionist required for formulation Expensive Labor intensive Chance of relapse at ~6-8 weeks (appreciated anecdotally for some cases) Finding a source of novel protein and being able to get it consistently Bioavailability has not been evaluated so need regular bloodwork and evaluation of physical exam parameters

Diet trials inevitably involve trial and error. Up to three different diets may be used before a response is seen. For large bowel diarrhea, a hydrolyzed or fiber-enriched diet should usually be the first diet choice and diet history can be used to guide which one should be trialed first. If fails first diet, then the other dietary strategy should be second choice.

Selecting a fiber-enriched diet

Selecting a fiber-enriched diet begins with evaluating the current and potential diets' total dietary fiber levels. The package labels are only required to show the crude fiber content, so it may be necessary to contact the manufacturer to obtain the total dietary fiber content.

There is no real consensus on what constitutes a low versus moderate versus high fiber diet, but the following table from Moreno et al., 2022 provides basic guidance.

Dietary fiber (in g/100 kcal)				
Fiber level	**Dogs**		**Cats**	
	Crude fiber	TDF	Crude fiber	TDF
Low	<1.0	<2.0	<0.5	<0.5
Moderate	1.0-2.0	2.0-4.0	0.5-1.0	1.0-2.5
High	>2.0	>4.0	>1.0	>2.5
Range	0.1-6.8	0.1-11.0	0.1-4.9	0.2-7.5

Ensuring owner compliance

The value of **owner education** cannot be underestimated when it comes to successful dietary trials. Set the stage by establishing the importance of the dietary trial as well as the trial-and-error approach that may be required to find the correct diet because every pet is different. Inform owners that the diet response is usually better than medications, both short-term as well as long-term. Provide the reasoning behind the diet recommendation. Inform pet owners of the

potential consequences of not following the directions, including the feeding of unapproved foods and treats.

Provide **clear recommendations** regarding the exact name of the recommended diet, dry or wet (or both), amount and frequency of feeding, length of time until reassessment, and allowed treats. Avoid generic recommendations: for example, provide the specific hydrolyzed diet name instead of telling them to choose one from a list because that creates the impression that all hydrolyzed diets are interchangeable when they are not. Provide the source of the diet to prevent owners from choosing something they think is the same but is not. Handouts and veterinary nurse/technician visits and follow-ups are valuable.

Use a **relationship-centred** approach focused on non-verbal cues, checking in with the client's thoughts, addressing their worries, using visuals for support, ensuring follow-up, and using forward booking to schedule rechecks so they are not overlooked or missed. For example, reassuring the client that lifelong feeding of the trial diet is not often needed.

Ensuring pet compliance

The pet's eating environment should be considered and modified if needed to optimize their acceptance of the diet. The pet's diet history may be helpful by signaling the pet's dietary preferences. Using dinner plates and leveraging social competition may be helpful, as well as timing of feeding (i.e., when the rest of the family is eating). A slow transition to the trial diet may be necessary, especially in cats. Other modifications to improve acceptance include warming, adding water, palatant enhancers, anti-nausea medications, analgesia, and altering the texture of the food.

If the pet is resisting a hydrolyzed diet, owners may be able to supplement with another food, such as low-fat cottage cheese, but the additional food should be kept to 10% or less of total daily calories to avoid unbalancing the main diet.

Assessing response and next steps

The diet should be fed exclusively for at least two weeks before assessing the response. Evaluation of the diet trial's efficacy is based on pet-related and owner-related factors. **Pet-related factors** include changes in body weight, body condition score, and GI signs. Validated tools such as CCECAI, CIBDAI, or FCEAI provide quantitative evaluation; full remission is reflected by >75% improvement in score, partial remission by 25-75% improvement, and <25% improvement indicates no remission.

With the exception of dogs with protein-losing enteropathy, which should remain on the effective diet long-term, the majority of pets that exhibit full remission can be slowly transitioned 12-14 weeks later to another diet. If only partial remission is observed, consider fiber supplementation and/or probiotic use before trying another diet. For cases exhibiting no remission, another diet trial is indicated. However, do not change diets just for the sake of changing diets; there needs to be justification for changing strategies.

Important **owner-related factors** include the owner's perception of the pet's percent improvement (keep in mind there may be a disjoint between the owner's perception and the disease index) and quality of life.

Putting the science into practice:

- A thorough diet history provides critical information that can optimize diet selection for dietary trials.
- Add fiber (psyllium is preferred) for persistent large bowel signs. If the owner likes the current diet, recommend supplemental fiber. If the response to fiber supplementation is insufficient, then transitioning to another diet with different strategy may be needed. If there is some response to the fiber supplementation, then consideration can be given to transitioning to a fiber-enriched diet.
- Before escalating to steroids, definitely try multiple diet trials.
- Give very specific diet instructions including the exact name of the recommended diet, dry or wet (or both), amount and frequency of feeding, length of time until reassessment, and allowed treats.
- Although case-dependent, typically 3-4 diet trials should be performed before ruling out food-responsive enteropathy. Recommended diet succession typically includes two hydrolyzed trials followed by one limited-ingredient or fiber-enriched diet.
- Client education and a relationship-centered approach to communication are critical to success.

References:

Allenspach, K., Wieland, B., Gröne, A., & Gaschen, F. (2007). Chronic enteropathies in dogs: Evaluation of risk factors for negative outcome. *Journal of Veterinary Internal Medicine*, *21*(4), 700–708. doi: 10.1111/j.1939-1676.2007.tb03011.x

Bandara, Y., Priestnall, S. L., Chang, Y., & Kathrani, A. (2023). Characterization of intestinal fibrosis in cats with chronic inflammatory enteropathy. *Journal of Veterinary Internal Medicine*, *37*(3), 936–947. doi: 10.1111/jvim.16688

Green, J., & Kathrani, A. (2022). Incidence of relapse of inflammatory protein-losing enteropathy in dogs and associated risk factors. *Journal of Veterinary Internal Medicine*, *36*(6), 1981–1988. doi: 10.1111/jvim.16561

Jergens, A. E., Crandell, J. M., Evans, R., Ackermann, M., Miles, K. G., & Wang, C. (2010). A clinical index for disease activity in cats with chronic enteropathy: Gastrointestinal disease activity index for cats. *Journal of Veterinary Internal Medicine*, *24*(5), 1027–1033. doi: 10.1111/j.1939-1676.2010.0549.x

Kathrani, A. (2021). Dietary and nutritional approaches to the management of chronic enteropathy in dogs and cats. *Veterinary Clinics of North America: Small Animal Practice*, *51*(1), 123–136. doi: 10.1016/j.cvsm.2020.09.005

Kratzer, G. R., Shepherd, M., Delaney, S. J., Winston, J. A., Rudinsky, A. J., & Parker, V. J. (2022). Home-cooked diets cost more than commercially prepared dry kibble diets for dogs with chronic enteropathies. *Journal of the American Veterinary Medical Association*, *260*(S3), S53–S60. doi: 10.2460/javma.22.07.0284

Mandigers, P. J. J., Biourge, V., Van Den Ingh, T. S. G. A. M., Ankringa, N., & German, A. J. (2010). A randomized, open-label, positively-controlled field trial of a hydrolyzed protein diet in dogs with chronic small bowel enteropathy: Hydrolyzed diets for canine chronic enteropathy. *Journal of Veterinary Internal Medicine*, *24*(6), 1350–1357. doi: 10.1111/j.1939-1676.2010.0632.x

Moreno, A. A., Parker, V. J., Winston, J. A., & Rudinsky, A. J. (2022). Dietary fiber aids in the management of canine and feline gastrointestinal disease. *Journal of the American Veterinary Medical Association*, *260*(S3), S33–S45. doi: 10.2460/javma.22.08.0351

Schramm, A., & Kook, P. H. (2022). A descriptive study on the extent of dietary information obtained during consultations at a veterinary teaching hospital. *Animals*, *12*(5), 661. doi: 10.3390/ani12050661

Switching from antibiotics to microbiota-friendly treatment in chronic diarrhea dogs – a case-based presentation

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A recent Swedish retrospective study of almost 100.000 consultations from 2020-2023 showed a mean yearly antibiotic prescription rate of 5.7% for canine gastroenteritis, including both acute and chronic cases (Ljunguist el al 2024, ECVIM abstract). Despite halving antibiotic use for canine gastroenteritis from 2020 to 2023, there has been NO increase in mortality rate. Increasing evidence shows that the negative effects of routine antibiotic use for canine gastroenteritis far outweigh the perceived positive effects, yet antibiotic use remains common in some countries. The following cases provide examples of how microbiome-centric approaches can improve management of GI conditions.

Meet Ebba

Ebba was a 1.3 year old short-haired Collie with diarrhea, intermittent vomiting, and hyporexia that started 9 months prior. Although the vomiting resolved after multiple diet changes, the diarrhea continued (fecal score 6-7). Treatments included multiple diets (including highly digestible GI, raw, and hydrolyzed diets), omeprazole, psyllium, metronidazole, prednisolone, multi-strain probiotic, and sucralfate.

Ebba's diet was supplemented with a multi-fiber prebiotic and the metronidazole dose was stopped, with excellent response. Three weeks later, the prednisone dose was tapered but the prebiotics, probiotics, and salmon-based hydrolyzed diet were continued. Ebba's clinical signs resolved and she maintained a normal fecal score and normal energy level. She exhibited one stress-induced relapse when she was taken to visit family in the city, but the issue resolved when she returned home.

What can Ebba teach us?

Ebba's case emphasizes that fiber isn't only for colitis. Beneficial fibers improve microbial diversity, reduce pH and the loss of amino acids, and increase short-chain fatty acid (SCFA) production. SCFAs can lower the cortisol stress-response in humans, and may have been a contributing factor for the improvement of Ebba's stress diarrhea. In a recent report (Alves et al., 2021), police dogs with diarrhea were administered daily psyllium, resulting in very good response in 50% of the dogs, good response in 40%, and a poor response in only 10% of the dogs. The effect lasted three weeks after the psyllium was discontinued; when clinical signs returned, the psyllium administration was resumed. The dogs' clinical signs resolved and they were maintained on fiber supplementation.

Meet Tyra

Tyra was a 4.5 year old intact female Shar pei with loose stool/diarrhea most days. She began vomiting a few months ago, and exhibits mild hyporexia and frequent defecation. Treatment has included a highly digestible GI diet, high fiber diet, and hydrolyzed diet as well as metronidazole. She responded to metronidazole every time, but relapsed within a few weeks. Bloodwork revealed low cobalamin, low folate, and low cholesterol levels.

Endoscopy showed moderate lymphocytic-plasmacytic enteritis and colitis with moderate villous atrophy. Her diet was changed to a novel protein diet, she was supplemented with cobalamin and folate, and methylprednisolone administration was initiated. She was doing well at recheck, and maintained on the novel diet, methylprednisolone and cobalamin. She was kept on very low-dose methylprednisolone for rest of her life and exhibited very low disease activity. She was euthanized at 15 years for severe osteoarthritis and cognitive dysfunction syndrome.

What can Tyra teach us?

Tyra fits the traditional definition of antibiotic-responsive enteropathy (ARE), but antibiotics should be the LAST option. Relapses are common when antibiotics are administered for chronic

enteropathy: studies have reported 86% relapse within 30 days (Westermarck et al, 2005), 88% relapse within 2 months (Kilpenin et al, 2011), and 100% relapse within 12 months (Allenspach et al, 2016). This sets up a vicious cycle of repeated antibiotic use.

Diarrhea that responds to antibiotics does NOT exclude the possibility of a response to corticosteroids or immunomodulatory treatment. There are no studies to date comparing immunomodulatory treatment versus antibiotics, so the true prevalence of ARE is unknown. A recent retrospective study (Hodel et al, 2024) evaluated dogs with chronic enteropathy with a minimum of 1 year of follow-up and with ARE defined as cases in which neither diet nor immunomodulatory treatment were successful: based on these criteria, none of the 60 dogs had ARE.

Owners understandably want the "quick fix" for diarrhea, and this drives requests for antibiotics because they perceive that antibiotics are effective. Education is the key to changing this demand. One allegory that may help is to compare antibiotics to wetting one's pants – they may provide a perception of instant relief, but at a longer-term cost (i.e., walking around with wet pants). Emphasize good versus bad bacteria in the gut, and how antibiotics create an imbalance that favors the bad bacteria. Acknowledge that we now know better when it comes to antibiotic use for gastrointestinal disease.

Meet Moltas

Moltas was a 5 year old intact male German Shepherd with lifelong chronic, partially refractory diarrhea along with regurgitation, atopic dermatitis, pyoderma, and chronic otitis. He was somewhat stable on high-dose corticosteroids, but minimal or no effects were observed with azathioprine, cyclosporine, or diet trials (2 highly digestible GI diets, novel protein, fiber-rich, and partial results with hydrolyzed soy). Clinical improvement was observed with chlorambucil, and he would respond to tylosin or metronidazole during flare-ups but the flare-ups were becoming more frequent. He exhibited abdominal pain on palpation and bloodwork showed low albumin and total protein. Fecal evaluation was negative.

He was treated with budesonide, methlyprednisolone, chlorambucil, and cobalamin supplementation. Three fecal microbiota transplantations (FMTs) were performed at 10-20 day intervals. His regurgitation stopped after the first FMT. His feces improved and he was more playful after the second FMT. Following the third FMT, his diarrhea and abdominal pain resolved and his albumin, protein and cobalamin levels normalized. On follow-up, the owners reported he would have mild flare-ups every 3 months or so, but they only lasted 1-2 days and no antibiotics were administered. Almost two years later, he developed a more severe flare-up that was managed effectively with another course of three FMTs.

What can Moltas teach us?

It's a common misperception is that tylosin is acceptable to use because it's not used for people and therefore resistance isn't a concern. However, resistance genes can be shared with other macrolides such as erythromycin, azithromycin and this has been proven to occur in swine. There is a documented risk of household sharing of resistance genes: people with antimicrobial-resistant genes share resistance genes with their dogs. Therefore, although tylosin is not used in humans, its use still presents a public health risk.

FMT is an effective microbiome-centric approach for many gastrointestinal conditions. Anecdotally, oral FMT capsules are less effective. This may be the result of a much lower "dose" of microbiota compared to FMT using an enema. If the dog or cat has severe dysbiosis (as indicated by a very high Dysbiosis Index), the FMT's effect may not last as long so be prepared to add other treatments.

Meet Elvis

Elvis was a 5 year old neutered male Eurasier with a lifelong history of chronic diarrhea and hyporexia with poor response to GI diet or fiber-rich diet and partial response to metronidazole , corticosteroids, psyllium, and a mono-protein diet. The owners had tried to taper off the metronidazole several times, but Elvis' disease activity increased. No effect was observed with azathioprine. As long-term antibiotic prescriptions had recently become more restricted, a change in his management was necessary.

Olsalazine administration was begun and his metronidazole was tapered. Olsalazine (5-ASA) has local anti-inflammatory effect on colonic mucosa, and decreases cyclooxygenase and lipoxygenase production. It is a common therapy for ulcerative colitis in people. Approximately 6-8 weeks of administration are needed to achieve full effect. Potential side effects of olsalazine in dogs include keratoconjunctivitis sicca (KCS) that may require treatment, worsening of diarrhea (rare), and possibly increased liver enzymes or hepatitis.
Two months later, he was exhibiting lower disease activity and was maintained on olsalazine and prednisolone.

What can Elvis teach us?

Antibiotics can have a negative effect on the regulatory immune response by decreasing regulatory T-cells, which reduces immunologic control and increases susceptibility to colitis. This increases the risk of multiple chronic disorders, including inflammatory bowel disease (IBD) in people. Stefka et al. (2014) administered antibiotics to eliminate some clostridial clusters in mice, then exposed the mice to peanut antigens. The mice developed peanut intolerance. But when multiple Clostridial clusters were re-installed before peanut exposure, the mice were tolerant of the peanut antigens.

Putting the science into practice:

- Try multiple strategies before antibiotics for chronic enteropathy (granulomatous colitis excluded).
  - Multiple dietary changes, pre/probiotics, FMT, immunoregulatory treatment, etc.
- Just because a dog with chronic diarrhea responds to antibiotics, that doesn't mean it won't respond to other treatments.
- For dogs that commonly develop stress diarrhea, increase their fiber dose prior to the anticipated stress (e.g. boarding). Probiotics may also help.
- A healthy microbiome is needed for immunologic control, and antibiotics have a negative effect on immunologic control.
  - Don't create and feed the "Metronidazole Monster" or "Tylosin Troll."
  - Educate cat and dog owners on antimicrobial resistance.
- For owners insisting on a "quick fix" for their pet's diarrhea, particularly during the diet trial, alternatives to antibiotics include FMT, fiber, loperamide, and clay preparations.
- Steroids have minimal negative impact on the microbiome and can contribute to restoring the microbiota over time, but they should still be tapered to the lowest effective dose. If the dose cannot be tapered, this indicates the need for another approach.
  - Perform FMT before steroids when possible, especially in young animals.

References:

Allenspach, K., Culverwell, C., & Chan, D. (2016). Long-term outcome in dogs with chronic enteropathies: 203 cases. *Veterinary Record, 178*(15), 368. doi: 10.1136/vr.103557

Alves, J. C., Santos, A., Jorge, P., & Pitães, A. (2021). The use of soluble fibre for the management of chronic idiopathic large-bowel diarrhoea in police working dogs. *BMC Veterinary Research*, *17*(1), 100. doi: 10.1186/s12917-021-02809-w

Atarashi, K., Tanoue, T., Shima, T., Imaoka, A., Kuwahara, T., Momose, Y., Cheng, G., Yamasaki, S., Saito, T., Ohba, Y., Taniguchi, T., Takeda, K., Hori, S., Ivanov, I. I., Umesaki, Y., Itoh, K., & Honda, K. (2011). Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science*, *331*(6015), 337–341. doi: 10.1126/science.1198469

Belas, A., Menezes, J., Gama, L. T., & Pomba, C. (2020). Sharing of clinically important antimicrobial resistance genes by companion animals and their human household members. *Microbial Drug Resistance*, *26*(10), 1174–1185. doi: 10.1089/mdr.2019.0380

Dalile, B., Verliet, B., Bergonzelli, G., Verbeke, K., & Van Oudehove, L. (2020). Colon-delivered short-chain fatty acids attenuate the cortisol response to psychosocial stress in healthy men: a randomized, placebo-controlled trial. *Neuropsychopharmacology, 45*(13), 2257-2266. doi: 10.1038/s41386-020-0732-x

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*(11), 589–599. doi: 10.1111/jsap.12588

Hanifeh, M., Scarsella, E., Rojas, C. A., Ganz, H. H., Huhtinen, M., Laine, T., & Spillmann, T. (2024). Oral fecal microbiota transplantation in dogs with tylosin-responsive enteropathy—A proof-of-concept study. *Veterinary Sciences*, *11*(9), 439. doi: 10.3390/vetsci11090439

Hodel, S., Brugger, D., & Kook, P. H. (2024). Long-term evaluation of the initial response to therapy in 60 dogs with chronic inflammatory enteropathy. *Journal of Veterinary Internal Medicine*, *38*(5), 2444–2453. doi: 10.1111/jvim.17161

Jackson, C. R., Fedorka-Cray, P. J., Barrett, J. B., & Ladely, S. R. (2004). Effects of tylosin use on erythromycin resistance in Enterococci isolated from swine. *Applied and Environmental Microbiology*, *70*(7), 4205–4210. doi: 10.1128/AEM.70.7.4205-4210.2004

Jackson, M. I., & Jewell, D. E. (2019). Balance of saccharolysis and proteolysis underpins improvements in stool quality induced by adding a fiber bundle containing bound polyphenols to either hydrolyzed meat or grain-rich foods. *Gut Microbes*, *10*(3), 298–320. doi: 10.1080/19490976.2018.1526580

Jergens, A. E., & Heilmann, R. M. (2022). Canine chronic enteropathy—Current state-of-the-art and emerging concepts. *Frontiers in Veterinary Science*, *9*, 923013. doi: 10.3389/fvets.2022.923013

Kilpinen, S., Spillmann, T., Syrjä, P., Skrzypczak, T., Louhelainen, M., & Westermarck, E. (2011). Effect of tylosin on dogs with suspected tylosin-responsive diarrhea: A placebo-controlled, randomized, double-blinded, prospective clinical trial. *Acta Veterinaria Scandinavica*, *53*(1), 26. doi: 10.1186/1751-0147-53-26

Ljungquist, D., Andersson, A. M., Johansson, E., Tham, J., & Toresson, L. (2024, September 5). *How low can you go? Antibiotic use in Swedish dogs with gastoenteritis*. 34th ECVIM-CA Congress, Lyon, France.

Ljungquist, O., Ljungquist, D., Myrenås, M., Rydén, C., Finn, M., & Bengtsson, B. (2016). Evidence of household transfer of ESBL-/pAmpC-producing Enterobacteriaceae between humans and dogs – a pilot study. *Infection Ecology & Epidemiology*, *6*(1), 31514. doi: 10.3402/iee.v6.31514

Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S. C., Browne, A. J., Chipeta, M. G., Fell, F., Hackett, S., Haines-Woodhouse, G., Kashef Hamadani, B. H., Kumaran, E. A. P., McManigal, B., … Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *The Lancet*, *399*(10325), 629–655. doi: 10.1016/S0140-6736(21)02724-0

Stefka, A. T., Feehley, T., Tripathi, P., Qiu, J., McCoy, K., Mazmanian, S. K., Tjota, M. Y., Seo, G.-Y., Cao, S., Theriault, B. R., Antonopoulos, D. A., Zhou, L., Chang, E. B., Fu, Y.-X., & Nagler, C. R. (2014). Commensal bacteria protect against food allergen sensitization. *Proceedings of the National Academy of Sciences*, *111*(36), 13145–13150. doi: 10.1073/pnas.1412008111

Toombs-Ruane, L. J., Benschop, J., French, N. P., Biggs, P. J., Midwinter, A. C., Marshall, J. C., Chan, M., Drinković, D., Fayaz, A., Baker, M. G., Douwes, J., Roberts, M. G., & Burgess, S. A. (2020). Carriage of extended-spectrum-beta-lactamase- and AmpC beta-lactamase-producing *Escherichia coli* strains from humans and pets in the same households. *Applied and Environmental Microbiology*, *86*(24), e01613-20. doi: 10.1128/AEM.01613-20

Toresson, L., Spillmann, T., Pilla, R., Ludvigsson, U., Hellgren, J., Olmedal, G., & Suchodolski, J. S. (2023). Clinical effects of faecal microbiota transplantation as adjunctive therapy in dogs with chronic enteropathies—A retrospective case series of 41 dogs. *Veterinary Sciences*, *10*(4), 271. doi: 10.3390/vetsci10040271

Treese, J. S., Nolte, I., & Bach, J.-P. (2021). Chronic enteropathy in dogs – retrospective case analysis and results of an owner survey. *Tierärztliche Praxis Ausgabe K: Kleintiere / Heimtiere*, *49*(02), 94–103. doi: 10.1055/a-1354-0630

Velasquez-Manoff, M. (2015). The peacekeepers. *Nature*, *518*(7540), S3–S5. doi: 10.1038/518S3a

Volkmann, M., Steiner, J. M., Fosgate, G. T., Zentek, J., Hartmann, S., & Kohn, B. (2017). Chronic diarrhea in dogs – Retrospective study in 136 cases. *Journal of Veterinary Internal Medicine*, *31*(4), 1043–1055. doi: 10.1111/jvim.14739

Westermarck, E., Skrzypczak, T., Harmoinen, J., Steiner, J. M., Ruaux, C. G., Williams, D. A., Eerola, E., Sundbäck, P., & Rinkinen, M. (2005). Tylosin-responsive chronic diarrhea in dogs. *Journal of Veterinary Internal Medicine*, *19*(2), 177–186. doi: 10.1111/j.1939-1676.2005.tb02679.x

World Health Organization. (2021). 10 global health issues to track in 2021. Accessed October 29, 2024. https://www.who.int/news-room/spotlight/10-global-health-issues-to-track-in-2021

Microbiome shifts in canine and feline health and disease

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The gut microbiome is an immune and metabolic organ with far-reaching effects in the body. Dysbiosis – an imbalance in the gut microbiota – can result from changes in bacterial populations due to loss of normal taxa and/or overgrowth of potential pathobionts. The health effects of dysbiosis reflect changes in microbial function such as bile acid conversion, short-chain fatty acid production, and tryptophan-indole metabolism.

The microbiome can cause clinical signs in a number of ways, including:

- An increased number of bacteria in small intestine produces more bacterial metabolites that promote inflammation.
- Increased mucosa-adhering bacteria serve as an inflammatory stimulus.
- Excessive or abnormal substrates in the intestinal lumen promote increased bacterial fermentation, which leads to osmotic diarrhea.
- A shift in the microbiome reduces beneficial bacteria (e.g., *Peptacetobacter hiranonis* formerly named *Clostridium hiranonis*), resulting in a lack of secondary bile acids and consequential pathobiont overgrowth and inflammation.

Measuring dysbiosis

Next-generation sequencing (NGS) approaches have revolutionized gut microbiome research and can provide strain-level resolution. However, they are only semi-quantitative, suffer from high detection limits, and generate data that is compositional. Quantitative polymerase chain reaction (qPCR) with kit-based DNA extraction provides a reproducible and analytically sensitive approach to accurately quantify core bacteria in the intestine.

Despite individual variation among individual dogs and cats, each species has a core microbiome. Within the core species of the dog microbiome, alterations in seven bacterial taxa have been identified as key indicators of dysbiosis and are the drivers of the Dysbiosis Index (DI). Five of the 7 are beneficial, with reductions of these bacteria resulting in a shift toward dysbiosis. The remaining two are pathobionts, resulting in a shift toward dysbiosis when present in increased numbers.

Beneficial (Reduced numbers shift toward dysbiosis)	**Overgrowth** (Increased numbers shift toward dysbiosis)
Faecalibacterium Turicibacter Blautia Fusobacterium *P. hiranonis* (formerly *C. hiranonis*)	Streptococcus *E. coli*

A DI of 0 or less is considered normal. A DI between 0 and 2 in dogs indicates mild to moderate shifts in the microbiome, and a DI above 2 represents dysbiosis. The higher the DI, the more the microbiome shifts away from normal.

P. hiranonis is the main bile acid converter in dogs and cats, converting primary bile acids from the liver to the main secondary bile acids. Secondary bile acids are important signaling molecules. Dogs with decreased abundance of *P. hiranonis* have increased primary bile acids and decreased secondary bile acid concentration in feces, reflecting a lack of conversion. Decreased *P. hiranonis* is a major driver of dysbiosis.

GI disease and dysbiosis

Dysbiosis patterns vary across GI diseases. Acute diarrhea is rarely associated with an increased DI, although it may induce minor changes in individual taxa. Minor dysbiosis may be observed

with acute, severe necrotic enteritis, but most will return to normal within approximately 7 days. Increased *Clostridium perfringens* may be found in these cases, but this increase is self-limiting and does not require specific treatment.

Chronic enteropathy has a wide spectrum of DI, ranging from normal to severe, indicating different functional subsets of the disease. Chronic enteropathy (CE) may be associated with a disrupted mucus layer, more permeable intestinal barrier, and shortening of villi leading to malabsorption. Most of these abnormalities persist even in CE cases that have entered clinical remission, indicating a persistent underlying pathology. Carbohydrate metabolism is significantly different in a subset of dogs with CE and a normal DI compared to CE with dysbiosis. Similarly, the metabolism of cofactors and vitamins as well as amino acid metabolism are significantly different between CE with dysbiosis, CE without dysbiosis, and healthy controls.

Clostridium difficile is identified in approximately 35% of dogs with CE, and dogs positive for *C. difficile* showed a higher DI. However, the presence of *C. difficile* does not indicate specific treatment, and most dogs were food-responsive regardless of its presence. Of importance, *P. hiranonis* correlates negatively with *C. difficile*, as 87% of *C. difficile*-positive samples had reduced *P. hiranonis*.

Ideally, use of the DI will assist in staging the intestinal changes in order to allow better understanding of underlying pathologies, enable more tailored treatment, provide a better understanding of prognosis (ie, long-term changes in gut environment), and optimize selection of multimodal treatments.

Altering the microbiome, for better or worse

Diet induces changes in the fecal microbiota composition at a more individual taxonomic level, corresponding to the levels of protein or fat. However, diet has a small effect size with overlap between protein and fat levels at the overall community level. An offal-based diet (e.g., BARF) with a very high and undigestible protein level was associated with higher *C. perfringens* count and enterotoxin, and higher *E. coli* than a non-offal diet with lower protein levels, but still only induces a mild to moderate shift that can remain within reference limits.

Antibiotics exert significant effects on the gut microbiome and can induce severe dysbiosis. Metronidazole induces major microbiome changes over time in dogs. Broad-spectrum antibiotics can lead to long-lasting dysbiosis in cats. Healthy cats receiving amoxicillin clavulanate had dysbiosis for at least 14 days after discontinuation (longer in some cats, for at least 8 weeks).

The effects of antibiotics overpower the effects of diets on the microbiome. Healthy dogs on various diets were switched to a hydrolyzed protein diet and then administered metronidazole. Metronidazole administration induced a significant dysbiosis that took several weeks to resolve. Metronidazole also significantly reduced *P. hiranonis* and some dogs did not return to normal levels even weeks after discontinuing the metronidazole. In pet dogs the recovery was individual, whereas colony dogs recovered more quickly.

Omeprazole administration leads to a transient increase in the DI in healthy dogs due to increased *Streptococcus* (lactic acid bacteria) but does not change *P. hiranonis*.

Putting the science into practice:

- Functional shifts in the microbiome may be the cause or consequence, or likley both, of a disease process. For some conditions, dysbiosis appears to precede clinical disease.
- Acute enteritis is associated with a rapid recovery of function, whereas chronic enteropathy (CE) involves persistent dysfunction and metabolomic changes as well as persistent dysbiosis.
- Various overlapping pathologies exist for chronic enteropathy, which differ in extent between patients.
- A subset of CE patients has increased DI, increased markers of inflammation, malabsorption, and abnormal intestinal permeability.
- Severe dysbiosis is indicative of more severe ultrastructural and functional changes.
    - Despite clinical remission, most abnormalities persist, indicating persistent underlying pathology in chronic enteropathy
    - Need to address enteropathy AND dysbiosis, multimodal therapy; overlapping issues
- Early recognition of intestinal changes before clinical signs appear can improve case management of CE.

References:

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

Blake, A. B., Cigarroa, A., Klein, H. L., Khattab, M. R., Keating, T., Van De Coevering, P., ... & Suchodolski, J. S. (2020). Developmental stages in microbiota, bile acids, and clostridial species in healthy puppies. *Journal of Veterinary Internal Medicine, 34*(6), 2345-2356. doi: 10.1111/jvim.15928

Cagnasso, F., Suchodolski, J. S., Borrelli, A., Borella, F., Bottero, E., Benvenuti, E., Ferriani, R., Tolbert, M. K., Chen, C. C., Giaretta, P. R., & Gianella, P. (2024). Dysbiosis index and fecal concentrations of sterols, long-chain fatty acids and unconjugated bile acids in dogs with inflammatory protein-losing enteropathy. *Frontiers in microbiology*, *15*, 1433175. doi: 10.3389/fmicb.2024.1433175

Chaitman, J., Guard, B. C., Sarwar, F., Lidbury, J. A., Steiner, J. M., & Suchodolski, J. S. (2017). Fecal microbial transplantation decreases the dysbiosis index in dogs presenting with chronic diarrhea. *Journal of Veterinary Internal Medicine, 31*(4), 1287. doi: 10.1111/jvim.14778

Copple, B. L., & Li, T. (2016). Pharmacology of bile acid receptors: Evolution of bile acids from simple detergents to complex signaling molecules. *Pharmacological research*, *104*, 9-21. doi: 10.1016/j.phrs.2015.12.007

Doulidis, P. G., Galler, A. I., Hausmann, B., Berry, D., Rodríguez-Rojas, A., & Burgener, I. A. (2023). Gut microbiome signatures of Yorkshire Terrier enteropathy during disease and remission. *Scientific Reports*, *13*(1), 4337. doi: 10.1038/s41598-023-31024-w

Elzenbeck, I., Teichmann-Knorrn, S., Hartmann, K., Zablotski, Y., Suchodolski, J. & Dorsch, R. (2024). Evaluierung des Effekts von Renaltec bei Katzen mit chronischer Nierenerkrankung im IRIS Stage 2 und 3. *Tierärztliche Praxis Ausgabe K: Kleintiere / Heimtiere - Thieme, 52*(02), 130-131. doi: 10.1055/s-0044-1780475

Forry, S. P., Servetas, S. L., Kralj, J. G., Soh, K., Hadjithomas, M., Cano, R., ... & Jackson, S. A. (2024). Variability and bias in microbiome metagenomic sequencing: an interlaboratory study comparing experimental protocols. *Scientific Reports*, *14*(1), 9785. doi: 10.1038/s41598-024-57981-4

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), 12977. doi: 10.1038/s41598-022-17244-6

Hoisington, A. J., Stamper, C. E., Ellis, J. C., Lowry, C. A., & Brenner, L. A. (2024). Quantifying variation across 16S rRNA gene sequencing runs in human microbiome studies. *Applied Microbiology and Biotechnology*, *108*(1), 1-9. doi: 10.1007/s00253-024-13198-z

Honneffer, J. B., Guard, B. C., Steiner, J. M., & Suchodolski, J. S. (2015). Alterations in fecal metabolite profiles of dogs with chronic enteropathy. *Journal of Veterinary Internal Medicine, 29*(4), 1180-1181. doi: 10.1111/jvim.12609

Honneffer, J. B. (2017). Microbiota and metabolomic changes across various canine gastrointestinal diseases. Doctoral dissertation. Available at https://scholars.library.tamu.edu/vivo/display/n98a05e2f/Documents/View%20All

Jones, S. M., Gaier, A., Enomoto, H., Ishii, P., Pilla, R., Price, J., Suchodolski, J., Steiner, J. M., Papich, M. G., Messenger, K., & Tolbert, M. K. (2020). The effect of combined carprofen and omeprazole administration on gastrointestinal permeability and inflammation in dogs. *Journal of Veterinary Internal Medicine, 34*(5), 1886-1893. doi: 10.1111/jvim.15897

Kool, J., Tymchenko, L., Shetty, S. A., & Fuentes, S. (2023). Reducing bias in microbiome research: Comparing methods from sample collection to sequencing. *Frontiers in Microbiology*, *14*, 1094800. doi: 10.3389/fmicb.2023.1094800

Li, F., Liu, J., Maldonado-Gómez, M. X., Frese, S. A., Gänzle, M. G., & Walter, J. (2024). Highly accurate and sensitive absolute quantification of bacterial strains in human fecal samples. *Microbiome, 12*(1), 168. doi: 10.1186/s40168-024-01881-2

Li, Q., Larouche-Lebel, É., Loughran, K. A., Huh, T. P., Suchodolski, J. S., & Oyama, M. A. (2021). Gut dysbiosis and its associations with gut microbiota-derived metabolites in dogs with myxomatous mitral valve disease. *Msystems, 6*(2), 10-1128. doi: 10.1128/msystems.00111-21

Martínez-López, L. M., Pepper, A., Pilla, R., Woodward, A. P., Suchodolski, J. S., & Mansfield, C. (2021). Effect of sequentially fed high protein, hydrolyzed protein, and high fiber diets on the fecal microbiota of healthy dogs: a cross-over study. *Animal Microbiome, 3*, 1-12. doi: 10.1186/s42523-021-00101-8

Minamoto, Y., Otoni, C. C., Steelman, S. M., Büyükleblebici, O., Steiner, J. M., Jergens, A. E., & Suchodolski, J. S. (2015). Alteration of the fecal microbiota and serum metabolite profiles in dogs with idiopathic inflammatory bowel disease. *Gut microbes*, *6*(1), 33-47. doi: 10.1080/19490976.2014.997612

Phimister, F. D., Anderson, R. C., Thomas, D. G., Farquhar, M. J., Maclean, P., Jauregui, R., ... & Bermingham, E. N. (2024). Using meta-analysis to understand the impacts of dietary protein and fat content on the composition of fecal microbiota of domestic dogs (*Canis lupus familiaris*): A pilot study. *Microbiology Open, 13*(2), e1404. doi: 10.1002/mbo3.1404

Pilla, R., Gaschen, F. P., Barr, J. W., Olson, E., Honneffer, J., Guard, B. C., Blake, A. B., Villanueva, D., Khattab, M. R., AlShawaqfeh, M. K., Lidbury, J. A., Steiner, J. M., & Suchodolski, J. S. (2020). Effects of metronidazole on the fecal microbiome and metabolome in healthy dogs. *Journal of Veterinary Internal Medicine*, *34*(5), 1853–1866. doi: 10.1111/jvim.15871

Pilla, R., Guard, B. C., Blake, A. B., Ackermann, M., Webb, C., Hill, S., ... & Suchodolski, J. S. (2021). Long-term recovery of the fecal microbiome and metabolome of dogs with steroid-responsive enteropathy. *Animals*, *11*(9), 2498. doi: 10.3390/ani1109249

Pilla, R., Werner, M., Toresson, L., Lidbury, J., Steiner, J., Unterer, S., & Suchodolski, J. (2019). Evaluation of the recovery of the fecal microbiome and metabolome of dogs following acute diarrhea. Journal of Veterinary Internal Medicine, 33(5), doi: 10.1111/jvim.15597

Reisinger, A., Stübing, H., Ishii, P., Suchodolski, J., Unterer, S., Steiner, J., Lidbury, J., Hartmann, K., & Busch, K. (2022). Evaluation of intestinal barrier dysfunction in dogs with acute hemorrhagic diarrhea syndrome. Journal of Veterinary Internal Medicine, 36(6), 2349. doi: 10.1111/jvim.16541

Roume, H., Mondot, S., Saliou, A., Le Fresne-Languille, S., & Doré, J. (2023). Multicenter evaluation of gut microbiome profiling by next-generation sequencing reveals major biases in partial-length metabarcoding approach. *Scientific Reports*, *13*(1), 22593. doi: 10.1038/s41598-023-46062-7

Rowe, J. C., Summers, S. C., Quimby, J. M., & Winston, J. A. (2024). Fecal bile acid dysmetabolism and reduced ursodeoxycholic acid correlate with novel microbial signatures in feline chronic kidney disease. *Frontiers in Microbiology, 15*, 1458090. doi: 10.3389/fmicb.2024.1458090

Schmidt, M., Unterer, S., Suchodolski, J. S., Honneffer, J. B., Guard, B. C., Lidbury, J. A., ... & Kölle, P. (2018). The fecal microbiome and metabolome differs between dogs fed Bones and Raw Food (BARF) diets and dogs fed commercial diets. *PloS ONE, 13*(8), e0201279. doi: 10.1371/journal.pone.0201279

Stübing, H., Reisinger, A., Werner, M., Unterer, S., Suchodolski, J., Steiner, J., Lidbury, J., Hartmann, K., & Busch, K. (2022). Comparison of metronidazole versus a synbiotic for treating dogs with acute diarrhea. Journal of Veterinary Internal Medicine, 36(6), 2426.doi: 10.1111/jvim.16541

Suchodolski, J. (2023). Assessing and managing the gut microbiome in canine and feline practice. In *Purina Institute Handbook of Canine and Feline Clinical Nutrition* (2nd ed., pp. 113–118). Embark Consulting Group, LLC.

Sung, C.-H., Pilla, R., Chen, C.-C., Ishii, P. E., Toresson, L., Allenspach-Jorn, K., Jergens, A. E., Summers, S., Swanson, K. S., Volk, H., Schmidt, T., Stuebing, H., Rieder, J., Busch, K., Werner, M., Lisjak, A., Gaschen, F. P., Belchik, S. E., Tolbert, M. K., ... Suchodolski, J. S. (2023). Correlation between targeted qPCR assays and untargeted DNA shotgun metagenomic sequencing for assessing the fecal microbiota in dogs. *Animals*, *13*(16), 2597. doi: 10.3390/ani13162597

Toresson, L., Spillmann, T., Pilla, R., Ludvigsson, U., Hellgren, J., Olmedal, G., & Suchodolski, J. S. (2023). Clinical effects of faecal microbiota transplantation as adjunctive therapy in dogs with chronic enteropathies—A retrospective case series of 41 dogs. *Veterinary Sciences*, *10*(4), 271. doi: 10.3390/vetsci10040271

Torres, J., Burisch, J., Riddle, M., Dubinsky, M., & Colombel, J.-F. (2016). Preclinical disease and preventive strategies in IBD: Perspectives, challenges and opportunities. *Gut*, *65*(7), 1061–1069. doi: 10.1136/gutjnl-2016-311785

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), 1-5. doi: 10.1038/nmicrobiol.2016.177

Vecchiato, C., Erba, D., Sportelli, F., Delsante, C., Alonzo, M., Pinna, C., Sabetti, M., Suchodolski, J., Pilla, R., Giaretta, P., Pietra, M., Biagi, G., & Procoli, F. (2023). Fecal microbial transplantation effect on clinical outcome and fecal microbiota and metabolome in dogs with chronic enteropathy refractory to diet. *Research Communications of the 33rd ECVIM-CA Congress*, 743. doi: 10.1111/jvim.16930

Vestergaard, M. V., Allin, K. H., Poulsen, G. J., Lee, J. C., & Jess, T. (2023). Characterizing the pre-clinical phase of inflammatory bowel disease. *Cell Reports Medicine*, *4*(11). doi: 10.1016/j.xcrm.2023.101263

Wang, S., Martins, R., Sullivan, M. C., Friedman, E. S., Misic, A. M., El-Fahmawi, A., De Martinis, E. C. P., O'Brien, K., Chen, Y., Bradley, C., Zhang, G., Berry, A. S. F., Hunter, C. A., Baldassano, R. N., Rondeau, M. P., & Beiting, D. P. (2019). Diet-induced remission in chronic enteropathy is associated with altered microbial community structure and synthesis of secondary bile acids. *Microbiome*, *7*(1), 126. doi: 10.1186/s40168-019-0740-4

Werner, M., Ishii, P. E., Pilla, R., Lidbury, J. A., Steiner, J. M., Busch-Hahn, K., … & Suchodolski, J. S. (2023). Prevalence of *Clostridioides difficile* in canine feces and its association with intestinal dysbiosis. *Animals, 13*(15), 2441. doi: 10.3390/ani13152441

Witkowski, M., Weeks, T. L., & Hazen, S. L. (2020). Gut microbiota and cardiovascular disease. *Circulation research, 127*(4), 553-570. doi: 10.1161/CIRCRESAHA.120.316242

Zheng, J., Sun, Q., Zhang, M., Liu, C., Su, Q., Zhang, L., … & Ng, S. C. (2024). Noninvasive, microbiome-based diagnosis of inflammatory bowel disease. *Nature Medicine*, 1-13. doi: 10.1038/s41591-024-03280-4

Žiga, G. (2016). Evaluation of the therapeutic potential of restoring gastrointestinal homeostasis by a fecal microbiota transplant in dogs. MS thesis. Available at https://www.ffa.uni-lj.si/fileadmin/datoteke/Knjiznica/magistrske/2016/Gerbec_Ziga_mag_nal_2016.pdf

Dietary tryptophan and the gut microbiome: Emerging approaches to optimize intestinal and systemic health

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Trytophan is an essential amino acid in dogs, cats, and humans. The majority (>90%) of dietary tryptophan is absorbed in the small intestine, but some (about 5%) enters the colon where it can be metabolized by gut microbiota. Interactions between tryptophan and the intestinal microbiota impact host animal health and disease.

Tryptophan not used for protein synthesis is metabolized in three primary metabolic pathways: kynurenine, serotonin, and microbial. The **kynurenine pathway** is an endogenous mammalian pathway that produces kynurenine and downstream catabolites and accounts for >90% of tryptophan catabolism. The basal kynurenine pathway occurs in the liver, but the extrahepatic pathway is upregulated by inflammation. This pathway generates neuroactive compounds that regulate the gut-brain axis to impact brain health and behavioral states. Conversion of tryptophan to kynurenine is increased by inflammatory cytokines and TLR activation by enteric microbiota. Kynurenine diffuses freely across the blood-brain barrier and is neuroprotective; however, downstream products of kynurenine are neurotoxic (e.g., quinolinic acid, an NMDA receptor agonist).

The **serotonin pathway** is also an endogenous mammalian pathway. Enteric microbiota and their metabolic products (e.g., SCFA) stimulate the intestinal enteroendocrine cells to synthesize and secrete serotonin (5-HT). More than 90% of all bodily serotonin is derived from the gut. Serotonin is secreted from the basal and apical aspects of the intestinal epithelium. Basal serotonin enters the portal and systemic circulation and impacts energy metabolism and development of liver and pancreas and signals via the vagus nerve to exert anti-anxiety and anti-depressive effects. Apical serotonin is secreted into the gut lumen and impacts the growth of commensal microbial communities. Serotonin interacts with the enteric and autonomic nervous systems, exerts anti-anxiety and anti-depressive actions, increases insulin secretion and promotes beta-cell survival, and promotes hepatocyte regeneration, stellate cell activation, and gluconeogenesis.

The **microbial indolic pathway** involves gut microbial conversion of tryptophan to microbial indole catabolites of tryptophan (MICTs; e.g., indolepropionic acid, indoleacetate, indolealdehyde), which are then absorbed into the submucosa and into the systemic circulation. Approximately 5% of dietary tryptophan is catabolized to MICTs. Numerous bacterial species can catabolize tryptophan to MICTs. MICTs regulate intestinal homeostasis, immune responses, organ system health, and energy metabolism. The MICT indolepropionic acid (IPA) has anti-fibrotic, intestinal homeostasis, anti-inflammatory, anti-oxidant, neuroprotective, anti-cancer, and anti-obesity effects. IPA promotes mucosal barrier function and modulates immune responses in canine colonocytes and ameliorates TNFα-induced barrier dysfunction (by decreasing intestinal permeability) and decreased IL-10RA expression. Bacteria associated with eubiosis (normal microbial balance) promote conversion of tryptophan to MICTs, while bacteria associated with dysbiosis promote conversion of tryptophan to indole. Indole can be metabolized in the liver to indoxyl sulfate, which is nephrotoxic and known to promote the progression of chronic kidney disease (CKD).

Serum MICT concentrations are decreased in cats with chronic inflammatory enteropathy (CIE) and low-grade intestinal T-cell lymphoma compared to healthy controls and are inversely correlated with feline chronic enteropathy activity indices. These findings are similar to those found in humans with IBD.

Dietary tryptophan has variable impacts on canine behavior. Some studies show no significant impact on behavior, but others showed reduced aggression/anxiety. However, because

supplements often contain other active ingredients, it is not possible to isolate the impact of each ingredient.

Dietary tryptophan supplementation increases blood serotonin concentrations and improves fecal quality scores in dogs. A combination of pectin fiber and tryptophan reduces microbial production of indole and promotes microbial production of other MICTs (indolelactate, indolepropionate) in the presence of tryptophan-catabolizing bacteria in vitro. More research is needed to understand the impact of tryptophan metabolism on gastrointestinal and systemic health in dogs and cats.

Human and lab animal studies show the promise of therapeutic interventions targeting tryptophan metabolism in the treatment of gastrointestinal, systemic, and behavioral disorders but have not yet been investigated for use in dogs and cats. Therapeutic manipulation of MICT synthesis is possible with targeted dietary interventions and may provide opportunities to shift tryptophan metabolism away from indole and optimize production of other MICTs. *L. reuteri* produces MICTs that protect against inflammation and mitigate dysbiosis in a rodent colitis model. Synbiotics and postbiotics may also offer benefits. Exogenous administration of IPA ameliorated colitis and reduced inflammatory cytokines in mice. Pharmaceutical interventions targeting indole-producing tryptophanase can reduce indole and indoxyl sulfate production.

Putting the science into practice:

- Tryptophan metabolism through the kynurenine, serotonin, and microbial indolic pathways is important to maintaining gastrointestinal, systemic, and psychologic/behavioral health.
- Therapeutic interventions targeting tryptophan metabolism are promising for treatment of gastrointestinal, systemic, and behavioral health.
- There are commercial diets that include tryptophan, but there is limited evidence to date to support its supplementation above baseline inclusion. Caution is advised when feeding these diets to pets with renal insufficiency due to the risk of indole compounds.

References:

Alexeev, E. E., Lanis, J. M., Kao, D. J., Campbell, E. L., Kelly, C. J., Battista, K. D., Gerich, M. E., Jenkins, B. R., Walk, S. T., Kominsky, D. J., & Colgan, S. P. (2018). Microbiota-derived indole metabolites promote human and murine intestinal homeostasis through regulation of interleukin-10 receptor. *The American Journal of Pathology*, *188*(5), 1183–1194. doi: 10.1016/j.ajpath.2018.01.011

Baumgart, M., Dogan, B., Rishniw, M., Weitzman, G., Bosworth, B., Yantiss, R., Orsi, R. H., Wiedmann, M., McDonough, P., Kim, S. G., Berg, D., Schukken, Y., Scherl, E., & Simpson, K. W. (2007). Culture independent analysis of ileal mucosa reveals a selective increase in invasive *Escherichia coli* of novel phylogeny relative to depletion of Clostridiales in Crohn's disease involving the ileum. *The ISME Journal*, *1*(5), 403–418. doi: 10.1038/ismej.2007.52

Chevalier, G., Laveissière, A., Desachy, G., Barnich, N., Sivignon, A., Maresca, M., Nicoletti, C., Di Pasquale, E., Martinez-Medina, M., Simpson, K. W., Yajnik, V., Sokol, H., MOBIDIC Study Investigators, Adegbamigbe, T., Ahmad, T., Arnott, I., Bouhnik, Y., Carbonnel, F., Colombel, J.-F., … Bonny, C. (2021). Blockage of bacterial FimH prevents mucosal inflammation associated with Crohn's disease. *Microbiome*, *9*(1), 176. doi: 10.1186/s40168-021-01135-5

Craven, M., Egan, C. E., Dowd, S. E., McDonough, S. P., Dogan, B., Denkers, E. Y., Bowman, D., Scherl, E. J., & Simpson, K. W. (2012). Inflammation drives dysbiosis and bacterial invasion in murine models of ileal Crohn's disease. *PLoS ONE*, *7*(7), e41594. doi: 10.1371/journal.pone.0041594

Del Carro, A., Corrò, M., Bertero, A., Colitti, B., Banchi, P., Bertolotti, L., & Rota, A. (2022). The evolution of dam-litter microbial flora from birth to 60 days of age. *BMC Veterinary Research*, *18*(1), 95. doi: 10.1186/s12917-022-03199-3

Dogan, B., Zhang, S., Kalla, S. E., Dogan, E. I., Guo, C., Ang, C. R., & Simpson, K. W. (2020). Molecular and phenotypic characterization of *Escherichia coli* associated with granulomatous colitis of Boxer dogs. *Antibiotics*, *9*(9), 540. doi: 10.3390/antibiotics9090540

Ghosh, T. S., Shanahan, F., & O'Toole, P. W. (2022). The gut microbiome as a modulator of healthy ageing. *Nature Reviews Gastroenterology & Hepatology*, *19*(9), 565–584. doi: 10.1038/s41575-022-00605-x

Gibson, J. F., Parker, V. J., Howard, J. P., Snell, C. M., Cross, E. W., Pagliughi, L. B., Diaz-Campos, D., Winston, J. A., & Rudinsky, A. J. (2022). *Escherichia coli* pathotype contamination in raw canine diets. *American Journal of Veterinary Research*, *83*(6), ajvr.21.10.0166. doi: 10.2460/ajvr.21.10.0166

Gookin, J. L., Strong, S. J., Bruno-Bárcena, J. M., Stauffer, S. H., Williams, S., Wassack, E., Azcarate-Peril, M. A., Estrada, M., Seguin, A., Balzer, J., & Davidson, G. (2022). Randomized placebo-controlled trial of feline-origin *Enterococcus hirae* probiotic effects on preventative health and fecal microbiota composition of fostered shelter kittens. *Frontiers in Veterinary Science*, *9*, 923792. doi: 10.3389/fvets.2022.923792

Graboski, A. L., Kowalewski, M. E., Simpson, J. B., Cao, X., Ha, M., Zhang, J., Walton, W. G., Flaherty, D. P., & Redinbo, M. R. (2023). Mechanism-based inhibition of gut microbial tryptophanases reduces serum indoxyl sulfate. *Cell chemical biology*, *30*(11), 1402–1413.e7. doi: 10.1016/j.chembiol.2023.07.015

Gueta, R., Hader, C., Mueller, A. S., Heusinger, T., & Mueller E. (2019). Prevalence of diarrheagenic *Escherichia coli* bacteria in fecal samples from dogs showing signs of gastrointestinal disorders and a clinically healthy control group. *JSMC Microbiololgy*, *1*, 5.

Janeczko, S., Atwater, D., Bogel, E., Greiter-Wilke, A., Gerold, A., Baumgart, M., Bender, H., McDonough, P. L., McDonough, S. P., Goldstein, R. E., & Simpson, K. W. (2008). The relationship of mucosal bacteria to duodenal histopathology, cytokine mRNA, and clinical disease activity in cats with inflammatory bowel disease. *Veterinary Microbiology*, *128*(1–2), 178–193. doi: 10.1016/j.vetmic.2007.10.014

Kittana, H., Gomes-Neto, J. C., Heck, K., Juritsch, A. F., Sughroue, J., Xian, Y., Mantz, S., Segura Muñoz, R. R., Cody, L. A., Schmaltz, R. J., Anderson, C. L., Moxley, R. A., Hostetter, J. M., Fernando, S. C., Clarke, J., Kachman, S. D., Cressler, C. E., Benson, A. K., Walter, J., & Ramer-Tait, A. E. (2023). Evidence for a causal role for *Escherichia coli* strains identified as adherent-invasive (AIEC) in intestinal inflammation. *mSphere*, *8*(2), e00478-22. doi: 10.1128/msphere.00478-22

Li, H., Limenitakis, J. P., Fuhrer, T., Geuking, M. B., Lawson, M. A., Wyss, M., Brugiroux, S., Keller, I., Macpherson, J. A., Rupp, S., Stolp, B., Stein, J. V., Stecher, B., Sauer, U., McCoy, K. D., & Macpherson, A. J. (2015). The outer mucus layer hosts a distinct intestinal microbial niche. *Nature Communications*, *6*(1), 8292. doi: 10.1038/ncomms9292

Manchester, A. C., Dogan, B., Guo, Y., & Simpson, K. W. (2021). *Escherichia coli* -associated granulomatous colitis in dogs treated according to antimicrobial susceptibility profiling. *Journal of Veterinary Internal Medicine*, *35*(1), 150–161. doi: 10.1111/jvim.15995

Morgan, X. C., Miller, M. L., Loftus, J. P., Frederick, C. E., Wakshlag, J. J., Zhang, S., Rishniw, M., & Simpson, K. W. (2024, September 5-7). *Limited ingredient diets remodel the microbiome and metabolome of dogs with food-responsive enteropathy*. [Conference presentation]. ECVIM 2024, Lyon, France.

Osbaldiston, G. W., & Stowe, E. C. (1971). Microflora of alimentary tract of cats. *American Journal of Veterinary Research, 32*(9), 1399-1405.

Pilla, R., Gaschen, F. P., Barr, J. W., Olson, E., Honneffer, J., Guard, B. C., Blake, A. B., Villanueva, D., Khattab, M. R., AlShawaqfeh, M. K., Lidbury, J. A., Steiner, J. M., & Suchodolski, J. S. (2020). Effects of metronidazole on the fecal microbiome and metabolome in healthy dogs. *Journal of Veterinary Internal Medicine*, *34*(5), 1853–1866. doi: 10.1111/jvim.15871

Puño-Sarmiento, J., Medeiros, L., Chiconi, C., Martins, F., Pelayo, J., Rocha, S., Blanco, J., Blanco, M., Zanutto, M., Kobayashi, R., & Nakazato, G. (2013). Detection of diarrheagenic Escherichia coli strains isolated from dogs and cats in Brazil. *Veterinary Microbiology*, *166*(3–4), 676–680. doi: 10.1016/j.vetmic.2013.07.007

Roager, H.M., & Licht, T.R. (2018). Microbial tryptophan catabolites in health and disease. *Nature Communications,* 9, 3294. doi: 10.1038/s41467-018-05470-4

Safety and efficacy of EcoActive on intestinal adherent invasive *E. Coli* in patients with inactive Crohn's disease ClinicalTrials.gov ID NCT03808103

Schmidt, V. M., Pinchbeck, G., McIntyre, K. M., Nuttall, T., McEwan, N., Dawson, S., & Williams, N. J. (2018). Routine antibiotic therapy in dogs increases the detection of antimicrobial-resistant faecal *Escherichia coli*. *Journal of Antimicrobial Chemotherapy*. doi: 10.1093/jac/dky352

Secher, T., Brehin, C., & Oswald, E. (2016). Early settlers: Which *E. coli* strains do you not want at birth? *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *311*(1), G123–G129. doi: 10.1152/ajpgi.00091.2016

Simpson, K. W., Dogan, B., Rishniw, M., Goldstein, R. E., Klaessig, S., McDonough, P. L., German, A. J., Yates, R. M., Russell, D. G., Johnson, S. E., Berg, D. E., Harel, J., Bruant, G., McDonough, S. P., & Schukken, Y. H. (2006). Adherent and Invasive *Escherichia coli* Is associated with granulomatous colitis in Boxer dogs. *Infection and Immunity*, *74*(8), 4778–4792. doi: 10.1128/IAI.00067-06

Simpson, K. W., Miller, M. L., Loftus, J. P., Rishniw, M., Frederick, C. E., & Wakshlag, J. J. (2023). Randomized controlled trial of hydrolyzed fish diets in dogs with chronic enteropathy. *Journal of Veterinary Internal Medicine*, *37*(6), 2334–2343. doi: 10.1111/jvim.16844

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*(3–4), 394–400. doi: 10.1016/j.vetmic.2009.11.002

U.S. Food and Drug Administration (2024) Outbreak investigation of E. coli 0157:H7: McDonalds Quarter Pounders (October 2024)

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

Wang, G., Fan, Y., Zhang, G., Cai, S., Ma, Y., Yang, L., Wang, Y., Yu, H., Qiao, S., & Zeng, X. (2024). Microbiota-derived indoles alleviate intestinal inflammation and modulate microbiome by microbial cross-feeding. *Microbiome,* 12, 59. doi: 10.1186/s40168-024-01750-y

Wang, S., Martins, R., Sullivan, M. C., Friedman, E. S., Misic, A. M., El-Fahmawi, A., De Martinis, E. C. P., O'Brien, K., Chen, Y., Bradley, C., Zhang, G., Berry, A. S. F., Hunter, C. A., Baldassano, R. N., Rondeau, M. P., & Beiting, D. P. (2019). Diet-induced remission in chronic enteropathy is associated with altered microbial community structure and synthesis of secondary bile acids. *Microbiome*, *7*(1), 126. doi: 10.1186/s40168-019-0740-4

Watson, V. E., Jacob, M. E., Flowers, J. R., Strong, S. J., DebRoy, C., & Gookin, J. L. (2017). Association of atypical enteropathogenic *Escherichia coli* with diarrhea and related mortality in kittens. *Journal of Clinical Microbiology*, *55*(9), 2719–2735. doi: 10.1128/JCM.00403-17

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: Small intestinal microbial communities in canine IBD. *FEMS Microbiology Ecology*, *66*(3), 579–589. doi: 10.1111/j.1574-6941.2008.00556.x

Young, W., Moon, C. D., Thomas, D. G., Cave, N. J., & Bermingham, E. N. (2016). Pre- and post-weaning diet alters the faecal metagenome in the cat with differences in vitamin and carbohydrate metabolism gene abundances. *Scientific Reports*, *6*(1), 34668. doi: 10.1038/srep34668

Zhang, S., Dogan, B., Guo, C., Herlekar, D., Stewart, K., Scherl, E. J., & Simpson, K. W. (2020). Short chain fatty acids modulate the growth and virulence of pathosymbiont *Escherichia coli* and host response. *Antibiotics*, *9*(8), 462. doi: 10.3390/antibiotics9080462

Zhang, S., Fu, J., Dogan, B., Scherl, E. J., & Simpson, K. W. (2018). 5-Aminosalicylic acid downregulates the growth and virulence of Escherichia coli associated with IBD and colorectal cancer, and upregulates host anti-inflammatory activity. *The Journal of Antibiotics*, *71*(11), 950–961. doi: 10.1038/s41429-018-0081-8

Zhang, S., Morgan, X., Dogan, B., Martin, F.-P., Strickler, S., Oka, A., Herzog, J., Liu, B., Dowd, S. E., Huttenhower, C., Pichaud, M., Dogan, E. I., Satsangi, J., Longman, R., Yantiss, R., Mueller, L. A., Scherl, E. J., Sartor, R. B., & Simpson, K. W. (2022). Mucosal metabolites fuel the growth and virulence of E. coli linked to Crohn's disease. *JCI Insight*, *7*(10), e157013. doi: 10.1172/jci.insight.157013

How the microbiome alters drug metabolism

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It has become general knowledge that many drugs affect the gut microbiome, but there is limited information to date on how the gut microbiome impacts the disposition of administered drugs.

Drugs that have been investigated for their impact on the microbiome in dogs include antibiotics, proton pump inhibitors, steroids, phenobarbital, anthelmintics, and chemotherapeutics. The list is shorter in cats and includes antibiotics, proton pump inhibitors, and antifungals.

PharmacoMICROBIOMICS is the study of the microbiome determinants of the different drug responses between individuals. Microbes impact drug pharmacokinetics and pharmacodynamics, and individual microbe-drug interactions have been identified Pharmacomicrobiomics is an important part of personalized medicine, which aims to optimize treatments for an individual based on their unique physiology.

Gut microbes have a huge metabolic capacity and can facilitate many different drug biotransformation reactions, some of which can only be performed by the microbes and not by the host/patient. The microbiome's activity may result in enhanced efficacy, reduced efficacy, enhanced toxicity, or a mixture of these effects. However, when dysbiosis is present, the microbiome's metabolic capacity is altered or reduced, which impacts an individual's ability to biotransform drugs.

Enhancing drug efficacy: prodrugs

Prodrugs are administered in their inactive form and are converted to an active metabolite that exerts the pharmacologic effect. The individual's capacity to metabolize the drug affects the conversion and, therefore, the effects of the drug. For example, sulfasalazine is converted by bacterial reduction in colon to 5-aminosalycylate (mesalazine), which is not absorbed and stays in the colon and has anti-inflammatory properties, and sulfapyridine, which is systemically absorbed and is a sulfonamide antibiotic with toxic potential.

Prodrug conversion can be impacted by altered gut microbial composition. Lovastatin, a cholesterol-lowering prodrug, is bioactivated to lovastatin hydroxy acid by gut microbes. Rats treated concurrently with lovastatin and antibiotics had lower active metabolite generation and systemic exposure, reflecting the impact of gut metabolism in drug absorption.

Decreasing drug efficacy: Inactivation

Microbial transformation of drugs can lead to reduced drug efficacy. For example, the cardiac glycoside digoxin is converted to an inactive metabolite (dihydro-digoxin) by the enzyme cardiac glycoside reductase (*cgr*). This enzyme is expressed by *Eggerthella lenta* bacterial strains that contain the *cgr* gene, which is only present in some individuals. Individuals carrying *cgr*+ *E. lenta* strains have decreased digoxin absorption and efficacy.

Increasing toxicity

Chloramphenicol provides an example of microbial drug transformation potentially increasing toxicity. The drug can be metabolized to metabolites that may be myelotoxic.

Reduction of metronidazole by the gut microbiome can produce N-(2-hydroxyethyl)-oxamic acid, which is a carcinogen.

Mixed effects

Non-steroidal anti-inflammatory drugs (NSAIDs) provide examples of mixed effects of microbial drug transformation because biotransformation in the gut increases both efficacy and toxicity. Carboxyl group-containing NSAIDs, like ketoprofen, carprofen, and robenacoxib, are

inactivated by glucuronidation in the liver prior to elimination in the bile. However, these metabolites can be reactivated by gut microbes and undergo enterohepatic recirculation, increasing drug efficacy. However, reactivation also makes the NSAIDs available for further biotransformation to toxic metabolites that mediate GI adverse effects. Alteration of the gut microbiome by antibiotic administration inhibits NSAID reactivation and thus may reduce toxicity, but will also reduce systemic exposure and, thus, drug efficacy.

Microbial effects on drug absorption

An altered microbiome may modulate drug absorption, possibly due to changes in the gut pH, transporter expression, and enterocyte drug metabolism.

A multi-strain probiotic altered the gut microbiome and shifted jejunal expression of Phase I enzymes (e.g., cytochrome p450 enzymes [CYPs]), Phase II enzymes (glutathione, sulfate, acetate, glucuronide conjugation), and transporters, leading to altered systemic exposure of multiple drugs including omeprazole.

Microbial effects on drug metabolism by the host/patient

Microbial products can competitively inhibit hepatic drug-metabolizing enzymes. For example, acetaminophen is preferentially metabolized in the liver by sulfation or glucuronidation to produce inactive metabolites. However, the microbial product p-cresol is structurally similar to acetaminophen and is metabolized by these same pathways. Competition between acetaminophen and p-cresol metabolism can push acetaminophen metabolism away from sulfation and glucuronidation and toward CYP oxidation, which produces the toxic metabolite that causes liver injury.

Microbial products also influence hepatic enzyme expression. Products like secondary bile acids and indole metabolites stimulate the pregnane X receptor in the liver, which upregulates CYP expression and activity. Without a gut microbiome, animals have significantly reduced hepatic biotransformation capacity and altered drug pharmacokinetics.

Putting the science into practice:

- The GI microbiome alters drug metabolism through direct transformation by microbiota as well as indirect effects on host biotransformation.
- The microbiome contributes to inter-individual variation in pharmacokinetics and drug response.
- The relationships between drug, microbiome, and host/patient are complex.
- Modern techniques allow investigation of HOW these interactions occur, and further research will investigate interventions that can optimize drugs' beneficial effects while minimizing their adverse effects.

References:

Clayton, T. A., Baker, D., Lindon, J. C., Everett, J. R., & Nicholson, J. K. (2009). Pharmacometabonomic identification of a significant host-microbiome metabolic interaction affecting human drug metabolism. *Proceedings of the National Academy of Sciences*, *106*(34), 14728–14733. doi: 10.1073/pnas.0904489106

Haiser, H. J., Gootenberg, D. B., Chatman, K., Sirasani, G., Balskus, E. P., & Turnbaugh, P. J. (2013). Predicting and manipulating cardiac drug inactivation by the human gut bacterium *Eggerthella lenta*. *Science, 341*(6143), 295-298. doi: 10.1126/science.1235872

Jimenez, J., Arimura, G., Abou-Khalil, W., Isildar, M., & Yunis, A. (1987). Chloramphenicol-induced bone marrow injury: Possible role of bacterial metabolites of chloramphenicol. *Blood*, *70*(4), 1180–1185. doi: 10.1182/blood.V70.4.1180.1180

Matuskova, Z., Anzenbacherova, E., Vecera, R., Tlaskalova-Hogenova, H., Kolar, M., & Anzenbacher, P. (2014). Administration of a probiotic can change drug pharmacokinetics: Effect of *E. coli* Nissle 1917 on amidarone absorption in rats. *PLoS ONE*, *9*(2), e87150. doi: 10.1371/journal.pone.0087150

Yoo, D.-H., Kim, I. S., Van Le, T. K., Jung, I.-H., Yoo, H. H., & Kim, D.-H. (2014). Gut microbiota-mediated drug interactions between lovastatin and antibiotics. *Drug Metabolism and Disposition*, *42*(9), 1508–1513. doi: 10.1124/dmd.114.058354

Zemanová, N., Lněničková, K., Vavrečková, M., Anzenbacherová, E., Anzenbacher, P., Zapletalová, I., Hermanová, P., Hudcovic, T., Kozáková, H., & Jourová, L. (2021). Gut microbiome affects the metabolism of metronidazole in mice through regulation of hepatic cytochromes P450 expression. *PLOS ONE*, *16*(11), e0259643. doi: 10.1371/journal.pone.0259643

Zhang, Y., Chen, Z., Xiao, Y., Wu, T., Yang, H., Lui, Y., Zhou, R., ...Zhu, P. (2024). Effects of compound probiotics on pharmacokinetics of CYP450 probe drugs in rats. *Drug Metabolism and Disposition*, 52(11), 1297-1312. doi: 10.1124.dmd.124.001837

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