How the gut microbiome affects distal organs

Celeste Allaband, DVM, PhD
University of California, San Diego
San Diego, CA (USA)

Rob Knight, PhD
University of California, San Diego
San Diego, CA (USA)

Introduction

These proceedings will describe the microbiome and its role as an additional organ at the center of the crosstalk between diet and non-gastrointestinal organ systems. With increasing links to disease, new therapeutics, and rising public interest (for example, the recent Netflix documentary "Hack Your Health: The Secrets of Your Gut"), the microbiome is becoming increasingly important to the practice of both human and veterinary medicine. Just as DNA ancestry reports were first developed for people before expanding into the veterinary field, people began with exploring their microbiomes and are now becoming interested in their pets' microbiomes.

The Human Microbiome Project, together with citizen science platforms including the Microsetta Initiative (formerly the American Gut Project), are helping promote awareness of the microbiome and its influences on health and disease for humans. For pets, there are less robust open access databases for analysis and comparison, but new data is being generated all the time. Many companies are already trying to take advantage of this emerging field, but most aspects of microbiome science are still not ready to be used in a clinical capacity, especially in a veterinary context. However, clinical utility is getting closer every day, and being aware of developments will be important in the near future. Researchers in both academia and industry are constantly exploring new methods to modify the microbiome to improve health or treat disease, including drugs, diets, prebiotics, probiotics, postbiotics, and synbiotics. This session will explore the future promise, as well as limitations, of emerging research into the connection between different organ systems and the microbiome.

The basics

The microbiome consists of all the microscopic organisms present in a particular environment, which includes bacteria, archaea, fungi, protists (microbial eukaryotes), and viruses/phages; because the tools we have to assess the mycobiome (fungi) and virome are at earlier stages of development, however, most discussion of the microbiome refers to the bacteriome (bacteria). An essential part of every individual from every species, the microbiome is involved in nearly every aspect of health from birth to death. It is known to stimulate the development of the immune system early in life, aid in the digestion of food, and reduce pathogen colonization, among other functions.1 Some species, namely hindgut (e.g. horses) and foregut fermenters (e.g. cows), would literally die without their essential symbionts.

Each human or animal body is composed of many different areas with different microscopic environments. For example, the large intestine is very dense with bacteria and has a high proportion of liquid and nutrients present, so it is more like the amazon rainforest of body environments. The skin is more like a desert because it is very dry, exposed to the air, and exposed to UV light. Very different bacteria live in these different locations. For example, just like people, dogs have different microbiomes throughout the body.[11] Even locations that are close together - such as nasal, oral, and ear microbiomes - can still have radically different

microbial populations from each other. The microbiome can also rapidly respond to changes in its environmental factors, including – this includes diet modifications, shampoos, drugs of all kinds, antibiotics, and disease (Figure 1). In these Proceedings, we focus on the gut microbiome, because it is the environment most commonly linked to diet, drugs, and disease.

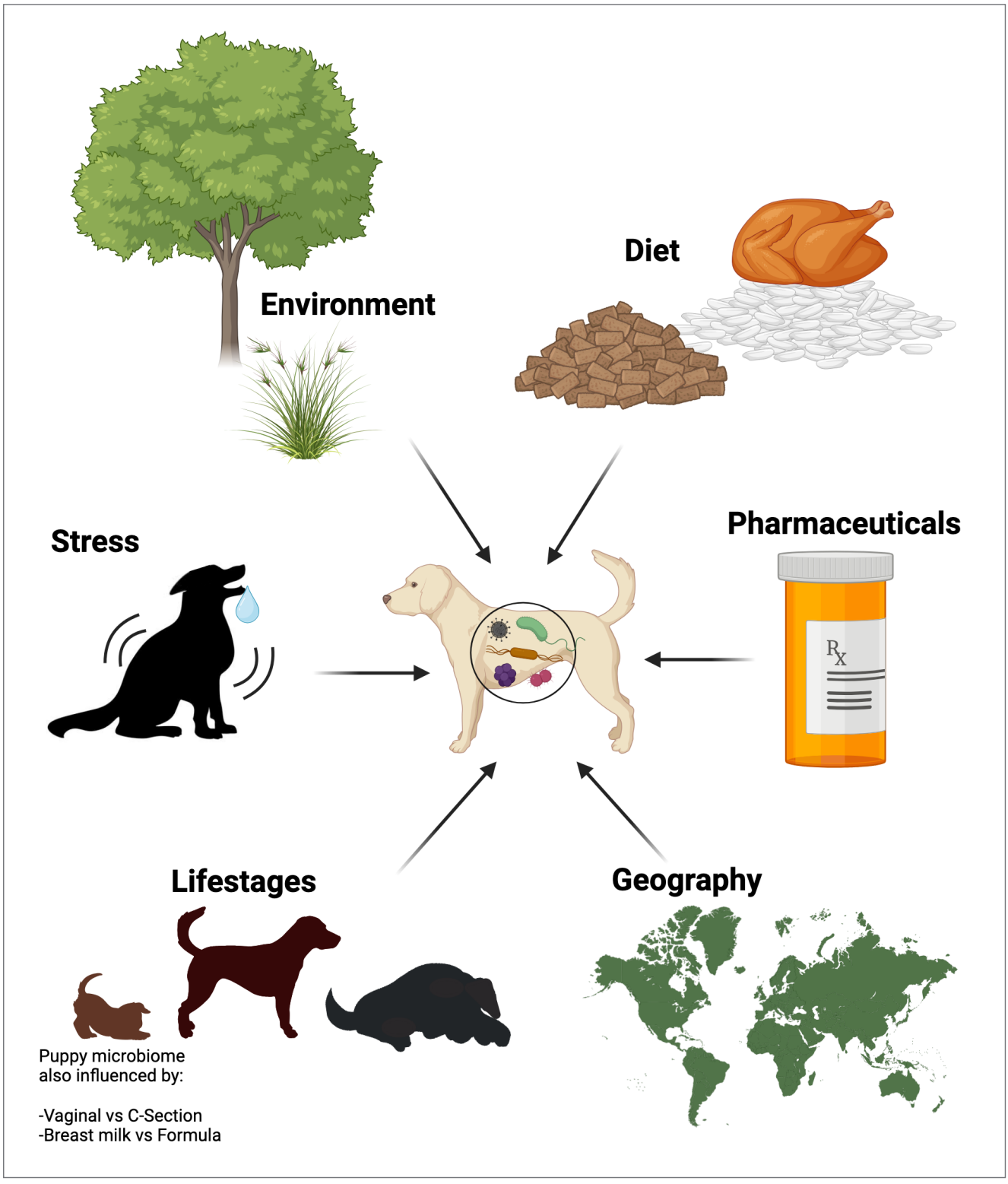

Figure 1: The many influences on the microbiome. The diversity of bacteria, fungi, and viruses within the microbiome is dynamic and highly responsive to a variety of factors. This includes diet, environmental factors, stress, life stages, geographical location, and pharmaceuticals.

Complicating the picture, but providing exciting opportunities for new diagnostics, the microbiome is exquisitely sensitive to a variety of host and environmental factors (pH, oxygen, immune system, diet, immune systems, and more). In fact, not only is the microbiome unique to a species or body region, it is unique to an individual. This individual specificity is even enabling the microbiome to begin to be used in forensic science to help solve crimes.[10] However, because there is no one, "normal" or "healthy" microbiome, microbiome science is not inherently well-suited to companion animal medicine, where we treat animals individually; examining the gut microbiome from a single fecal sample means nothing without data from other animals in the same environment to put it into context. Therefore, microbiome science is better suited to herd medicine, with many samples collected at once and used to contextualize each other - for example, by relating them to individual-level clinical phenotypes.

Imbalance in the normal microbiome, often called dysbiosis, has been challenging to define in detail due to the many different ways to assemble a microbiome in physiologically healthy individuals. The clinical symptom most commonly associated with gut microbial dysbiosis is diarrhea, but a wide variety of symptoms have been linked to "dysbiosis" by the method of demonstrating that symptomatic and asymptomatic individuals differ in their microbiome in one of many ways (composition, diversity, etc.). Differences in the microbiome have also been associated with diabetes mellitus, inflammatory bowel disease (IBD), neoplasia, hepatopathy, obesity, and many other chronic inflammatory conditions.

It can be easy for both owners and veterinarians to notice signs of dysbiosis, but it is often difficult to know what to do about it. Antibiotics are overprescribed for many conditions.[2,3] From human studies of over 100,000 healthy individuals[4], no one, perfect microbiome emerged, likely due to the many factors that can impact the microbiome. There have been efforts to try to help define the dog[5,6] and cat[7] healthy microbiomes, although none to a scale comparable for what has been done for humans, where even these attempts to date have been unsuccessful. Especially for cats, the effect of litter substrate choice on the ability to detect microbes, creating bias in the data, has not been rigorously explored. Therefore there is no normal value range to determine when something is abnormal. However, although there is no singular "normal" or "healthy" microbiome, some changes in the microbiome can clearly indicate a problem. There is currently a fecal quantitative polymerase chain reaction (qPCR) assay available from Texas A&M University, developed by Dr. Jan Suchodolski, that tests whether the balance of bacteria in the canine[8] and feline[9] intestinal tract resembles individuals known to have GI disease (generally inflammatory bowel disease (IBD) for dogs and chronic enteropathies for cats). The canine qPCR panel consists of eight bacterial groups: total bacteria, *Faecalibacterium*, *Turicibacter*, *Escherichia coli*, *Streptococcus*, *Blautia*, *Fusobacterium* and *Clostridium hiranonis*.[8] The feline qPCR panel consists of seven bacterial groups: total bacteria, *Bacteroides*, *Bifidobacterium*, *Clostridium hiranonis*, *Escherichia coli*, *Faecalibacterium*, *Streptococcus* and *Turicibacter*.[9] Sufficiently large differences in abundance from the reference healthy population in the panel bacteria has been defined as intestinal dysbiosis, and is predictive of gastrointestinal disease. The dysbiosis index correlates negatively with species richness - so a higher value indicates lower microbial diversity. Although these tests were developed to help identify patients with specific chronic gastrointestinal diseases, it is not explicitly diagnostic of any disease because many other factors, including drug administration, can affect the microbiome and must be considered when interpreting the results. There is also a surprising amount of molecular cross-talk between microbes and with their host. Because microbes "talk" in the language of molecules and receptors, they often talk to each other and to their host in ways not yet fully understood. Therefore, the examples of signals given here will likely greatly expand in the future.

The data

A typical Labrador retriever (approximately 30kg/66lbs) has about 13 trillion canine cells and about 18 trillion bacterial cells - about an equal amount. That is a lot of microscopic friends! Even a small (1g) stool sample from this patient contains about 100 billion microbes, each of which has its own genome. If we estimate that each microbe has a genome size of 5 million bases (conservatively), that is about 100,000 terabytes (TB) worth of data in every gram of stool.[12] Since an average new laptop has a 0.5 TB hard drive, that represents a truckload of information for just one small sample. Even with modern sequencing techniques and using a supercomputer, it would

take thousands of years to process and analyze all the data if we could sequence everything (in practice, far fewer sequences are collected with current technology, typically between 1000 and 100,000,000 per sample). Furthermore, we want to examine samples from many patients to build reference datasets, not just one.

In order to reduce the enormous job of sequencing microbial populations, a universal marker that is smaller and more manageable than the whole genome would be helpful. Fortunately, all bacteria have ribosomes for translating DNA into proteins. Because ribosomes are critical to cellular function, the DNA sequence that encodes them is always present and contains regions that are identical even across distantly-related species. However, the 16S subunit of the ribosome has changed enough during evolution to be useful for distinguishing different kinds of microbes from each other, although often at the genus level rather than the species or strain. The V4 region is the most commonly used for this purpose. Thus, this short DNA sequence has become a universal marker that can be linked to bacterial (and even archaeal) identification. This method is also independent of our ability to culture the bacteria in the laboratory, so many new bacteria that otherwise die in the presence of oxygen have been discovered as components of healthy and unhealthy gut microbiomes. 16S amplicon sequencing has been the most common method of evaluating the gut microbiome for about 20 years, but with increasing reference databases and decreasing sequencing costs, more options are available. Other targeted sequencing options include 18S or ITS amplicon sequencing, which get you information on eukaryotes (protists) or fungi, respectively, but the field is rapidly moving to untargeted approaches such as shotgun metagenomics.

One major limitation of amplicon-targeted sequencing microbiome analysis is that while we can identify the bacteria present, we do not know what exact role they play in the ecosystem. Additional analyses can help assess function, however. Metabolomics (all of the molecules in a sample), transcriptomics (all the bacterial or host RNA, which is much more transient than DNA and therefore responsive to current conditions or treatments), and proteomics (all of the proteins in a sample, and also a readout of transient gene expression states) all provide more information about changing biochemical pathways. This information can help improve interpretation and conclusions about what is going on in the microbial ecosystem. All of these methods are currently very expensive (with multi-million dollar instruments and costly reagents), and can be time-consuming. Another method of obtaining functional information is shotgun metagenomics (at the time of writing, this is most commonly collected as short read data, e.g. 150 base pair fragments, on the Illumina platform), which yields a random selection of all the DNA present in the sample, whether microbial or host. Although shotgun metagenomics is currently more expensive and difficult than using the 16S marker, sequencing costs are continuing to decrease and analysis methods are improving in both speed and ease of use. Emerging long read methods reading tens of thousands - or even millions - of bases, pioneered in sheep metagenomics, can obtain complete circular bacterial chromosomes in complex samples.[13]

The populations

While there is no single standard microbiome that all healthy individuals of the same species share, most mammals generally have a gut microbiome dominated by two phyla. Phylum Bacillota (formerly Firmicutes) are gram-positive cocci, many of which can produce endospores (examples: Bacillus, Clostridium). Phylum Bacteroidota (formerly Bacteroidetes) are gram-negative, generally rod-shaped bacteria that do not make endospores (examples: Bacteroides, Porphyromonas). Labeling these groups as "good" or "bad" is a gross over-generalization given each phylum contains thousands of different bacterial species to which these statements may not be true, or may be true only situationally. E.coli is a great example, with some strains known to cause food poisoning and enterohemorrhagic diarrhea (O157:H7), and other strains commercially sold as probiotics (Nissle1917).

Because the microbiome is a whole ecosystem of bacteria, we use ecological terms and calculations to discuss what is happening in sample populations. Diversity measurements are one of the key ways of assessing the microbiome. Alpha diversity refers to calculating and evaluating the bacterial population present within a single sample. For example, it has been found that dogs with IBD have lower alpha diversity than normal healthy dogs.[14] Although, caution should be taken in assuming more diversity is better. A desert has less diversity than a rainforest, but it is not less "healthy". For example, the skin is

less diverse than the intestinal tract, but that is normal for those environments. Beta diversity refers to the differences in bacteria present when samples are compared to each other. For example, skin samples from healthy cats with skin allergies overall had the same alpha diversity, but they had very different beta diversity.[15] From that information, we know that while they might have the same number of bacteria, the cats with allergic skin conditions have different types of bacteria from healthy controls. There are many different methods of calculating alpha and beta diversity that take different factors into account. For example, Jaccard distances use only the presence or absence of each taxon, while Bray-Curtis distances take the abundance of each bacteria into account. These are non-phylogenetic methods that treat all species as equally related to one another; phylogenetic methods such as UniFrac, which take evolution into account, are often useful for revealing biologically meaningful patterns.[16,17] Different methods for calculating diversity have different underlying theoretical bases and often provide complementary and differing insight into ecosystem function, including microbiomes.[18]

The most frequent method of making highly multivariate microbiome data interpretable in a useful visualization employs principal coordinate analysis (PCoA) to collapse the relationships between all the bacteria from each individual sample into a single point on a 2D or 3D plot. Points (samples) on the plot that are close together are more similar, and points that are far away from each other are more different. The distance can be related to a standardized scale of distances between points from known environments, e.g. physical environments, body habitats, or species. The clustering of points on the plot color-coded for different factors can give insights into what is influencing the microbiome.

There are an enormous number of potential confounding factors in any microbiome study, and it is important to control for both biological variability and differences in laboratory and bioinformatics methods, which can be large.[1,12] Biological variations that can influence samples are age, sex, diet, lifestyle, and even the time of day or the season.[1,12,19] Factors that influence results during processing samples into data include sample collection methods, shipping conditions, DNA extraction reagents, library construction, sequencing platform, and software processing.[1,12] Proper study design will account for these variables, but requires careful consideration of sample type and hypothesis. If the study is not well designed, analysis can take years and cost several hundred thousand dollars. It is always advisable to follow best practice recommendations.[1] It is also critical to keep and record all of the information related to these confounders in an excel-like file known as metadata (data that puts the DNA sequencing data in context, such as site or clinical information). Metadata is one of the most critical pieces of information that you create, especially if you are collaborating with someone else who is doing data analysis for you, because it is impossible to relate the microbiome data (e.g. the level of diversity, or of a particular taxon) to a phenotype or health condition that is not included in the metadata being analyzed.

The new organ

An organ is defined by the NIH as " a collection of tissues that structurally form a functional unit specialized to perform a particular function".[20] The gut microbiome is a collection of bacterial cells that form a functional unit that break down and metabolize food, drugs and other substances ingested by the host.[21] Therefore the gut microbiome plays a key part in ensuring that the host survives and thrives over time. Like many organs, the microbiome (and the metabolites it produces) also play a role in signaling the brain[22,23] and can induce and respond to host hormones.[24] Thus, the microbiome acts as another organ in the body and is integral to maintaining health in companion animals.[25]

Although the liver is often thought of as the main organ in drug metabolism due to its first-pass processing by cytochrome P450s and other enzymes, the microbiome (including the distinct oral, stomach and gut microbiomes) is arguably the first "organ" that experiences and can modify any oral medication given to patients. Many different drugs modify, or are modified by, the microbiome. The most obvious category is antibiotics, which can kill a pathogen but also obliterate or slow the growth of a huge number of normal commensal bacteria. The changes in the microbiome due to antibiotics are sometimes drastic and long-lasting, but it is not the only drug category that can affect and be affected by the microbiome. Ever wonder why some oral drugs work great in one patient but not in another? It may be due to that individual's microbiome. It is now known that the gut microbiome can alter absorption and metabolism of a wide variety of

pharmaceutical compounds[26], although most of this work has been done in humans or mice to date.

Like with other organs, the microbiome can also be transplanted. Fecal microbiota transfer (FMT) is where a fecal sample from a pre-screened healthy donor is collected and mixed into a saline solution, filtered to remove large debris, and placed in a patient via scope, jejunostomy tube, or enema. Administration orally or through an esophageal tube is generally not recommended because gastric fluids will kill most bacteria. FMT has seen a lot of recent successes treating human patients with chronic C. difficile infections. In veterinary medicine, FMT has successfully been used in disease states associated with dysbiosis, including canine parvovirus[27], canine inflammatory bowel disease[28,29], at least one case of feline ulcerative colitis[30], feline chronic digestive issues[31], chronic intermittent diarrhea in dolphins[32], and more. However, although this technique has been very successful in some cases, it still merits some caution. There have also been rare but notable failures, such as when a human patient died after receiving a fecal transplant that unknowingly contained a multi-drug resistant strain of E. coli.

We have known for a long time that the gut microbiome is also very responsive to dietary changes in many species.[33] This means that diet, probiotics, home-cooked diets, and treats really can have dramatic effects on digestion and metabolism. Microbial digestion or fermentation of food means that they interact with host-produced molecules, creating "postbiotics". Postbiotics are molecules/metabolites - such as short chain fatty acids and bile acids - that can have significant impacts on host health and disease processes. They can have both direct and indirect effects. For example, butyrate can have a direct anti-inflammatory effect on colonic epithelial cells. In addition, bile acids can act indirectly by changing the signals sent through receptors.

Diet has been shown to impact health significantly for every species examined so far. For example, in marmosets (non-human primate) a diet change has shown to significantly affect health, overall weight, and even fertility.[34] Diet has also been known to influence the fertility of other animals, including endangered southern white rhinoceros.[35,36] It has been repeatedly shown in many animal species that the dietary component that appears most critical for health is fiber, which is used for bacterial fermentation. This is true not just for species that perform large scale internal fermentation, such as rabbits and sheep, but also species with a simpler digestive tract. Changes to food can have outsized impacts. In fact, when manufacturers changed the processing of their extruded diets (kibble) to use low shear on extrusion resulting in longer fiber strands, dogs eating those diets showed improved fecal short chain fatty acid and bile acid profiles.[37,38]

Bile acids are one of the ways that bacteria can "talk" to the host through signals to organs all over the body. Bile acids are traditionally known for their ability to emulsify and help digest fats, but have recently been found to do so much more. They have a steroid core, similar to estrogen and cortisol. Primary bile acids are made by the liver, stored in the gallbladder (if the species has a gallbladder), secreted after a meal into the intestinal tract, reabsorbed into the bloodstream, and returned to the liver/gallbladder. This is termed enterohepatic circulation. There are receptors that detect bile acids in many parts of the GI tract and in other organs throughout the body.[39] The ability to signal organs all over the body is likely due to the transient time they spend in the bloodstream. There are many different bacteria that produce bile acid modifying enzymes that affect known receptors in many different ways. We are continuing to learn more and more about the many types of modifications possible and how they affect different receptors in the body.

The conclusion

The microbiome is increasingly recognized as an organ or pseudo-organ that influences numerous bodily functions. A frontier in veterinary medicine, it offers exciting future opportunities to enhance animal health and well-being – especially because unlike the host genome, the microbiome is easily modifiable by diet, drugs, and other interventions. Although challenges remain in data collection, analysis, and interpretation, the potential benefits are immense. By understanding the intricate interplay between the microbiome and the host and how they communicate with one another, we can develop targeted interventions, such as tailored diets, to optimize animal health. As research progresses, we can anticipate a future where microbiome-based therapies become standard practice, revolutionizing the care of our companion animals.

References:

1. Knight R, Vrbanac A, Taylor BC, et al. Best practices for analysing microbiomes. Nat Rev Microbiol 2018;16:410–422.
2. Del Solar Bravo RE, Sharman MJ, Raj J, et al. Antibiotic therapy in dogs and cats in general practice in the United Kingdom before referral. J Small Anim Pract 2023;64:499–506.
3. Llanos-Soto SG, Vezeau N, Wemette M, et al. Survey of perceptions and attitudes of an international group of veterinarians regarding antibiotic use and resistance on dairy cattle farms. Prev Vet Med 2021;188:105253.
4. McDonald D, Hyde E, Debelius JW, et al. American Gut: an Open Platform for Citizen Science Microbiome Research. Greene CS, ed. mSystems 2018;3:e00031-18, / msystems/3/3/msys.00031-18.atom.
5. Jha AR, Shmalberg J, Tanprasertsuk J, et al. Characterization of gut microbiomes of household pets in the United States using a direct-to-consumer approach. Clegg SR, ed. PLOS ONE 2020;15:e0227289.
6. Alessandri G, Milani C, Mancabelli L, et al. Metagenomic dissection of the canine gut microbiota: insights into taxonomic, metabolic and nutritional features. Environ Microbiol 2019;21:1331–1343.
7. Ganz HH, Jospin G, Rojas CA, et al. The Kitty Microbiome Project: Defining the Healthy Fecal "Core Microbiome" in Pet Domestic Cats. Vet Sci 2022;9:635.
8. AlShawaqfeh M, Wajid B, Minamoto Y, et al. A dysbiosis index to assess microbial changes in fecal samples of dogs with chronic inflammatory enteropathy. FEMS Microbiol Ecol 2017;93. Available at: http://academic.oup.com/femsec/article/doi/10.1093/femsec/fix136/4443197. Accessed August 14, 2024.
9. Sung C-H, Marsilio S, Chow B, et al. Dysbiosis index to evaluate the fecal microbiota in healthy cats and cats with chronic enteropathies. J Feline Med Surg 2022;24:e1–e12.
10. Metcalf JL, Xu ZZ, Bouslimani A, et al. Microbiome Tools for Forensic Science. Trends Biotechnol 2017;35:814–823.
11. Pereira AM, Clemente A. Dogs' Microbiome From Tip to Toe. Top Companion Anim Med 2021;45:100584.
12. Allaband C, McDonald D, Vázquez-Baeza Y, et al. Microbiome 101: Studying, Analyzing, and Interpreting Gut Microbiome Data for Clinicians. Clin Gastroenterol Hepatol 2019;17:218–230.
13. Bickhart DM, Kolmogorov M, Tseng E, et al. Generating lineage-resolved, complete metagenome-assembled genomes from complex microbial communities. Nat Biotechnol 2022;40:711–719.
14. Vázquez-Baeza Y, Hyde ER, Suchodolski JS, et al. Dog and human inflammatory bowel disease rely on overlapping yet distinct dysbiosis networks. Nat Microbiol 2016;1:16177.
15. Older CE, Diesel A, Patterson AP, et al. The feline skin microbiota: The bacteria inhabiting the skin of healthy and allergic cats. PloS One 2017;12:e0178555.
16. Lozupone C, Knight R. UniFrac: a New Phylogenetic Method for Comparing Microbial Communities. Appl Environ Microbiol 2005;71:8228–8235.
17. Hamady M, Knight R. Microbial community profiling for human microbiome projects: Tools, techniques, and challenges. Genome Res 2009;19:1141–1152.
18. Lozupone CA, Hamady M, Kelley ST, et al. Quantitative and Qualitative β Diversity Measures Lead to Different Insights into Factors That Structure Microbial Communities. Appl Environ Microbiol 2007;73:1576–1585.
19. Allaband C, Lingaraju A, Flores Ramos S, et al. Time of sample collection is critical for the replicability of microbiome analyses. Nat Metab 2024. Available at: https://www.nature.com/articles/s42255-024-01064-1. Accessed July 20, 2024.
20. National Human Genome Research Institute. US Dep Health Hum Serv Natl Inst Health 2024. Available at: https://www.genome.gov/genetics-glossary/Organ. Accessed August 14, 2024.
21. Baquero F, Nombela C. The microbiome as a human organ. Clin Microbiol Infect 2012;18:2–4.
22. Van De Wouw M, Schellekens H, Dinan TG, et al. Microbiota-Gut-Brain Axis: Modulator of Host Metabolism and Appetite. J Nutr 2017;147:727–745.
23. Hussain SS, Bloom SR. The regulation of food intake by the gut-brain axis: implications for obesity. Int J Obes 2013;37:625–633.
24. Evans JM, Morris LS, Marchesi JR. The gut microbiome: the role of a virtual organ in the endocrinology of the host. J Endocrinol 2013;218:R37–R47.
25. Suchodolski JS. Analysis of the gut microbiome in dogs and cats. Vet Clin Pathol 2022;50:6–17.
26. Wilson ID, Nicholson JK. Gut microbiome interactions with drug metabolism, efficacy, and toxicity. Transl Res 2017;179:204–222.
27. Pereira GQ, Gomes LA, Santos IS, et al. Fecal microbiota transplantation in puppies with canine parvovirus infection. J Vet Intern Med 2018;32:707–711.
28. Bottero E., Benvenuti E., Ruggiero P. Faecal microbiota transplantation in 16 dogs with idiopathic inflammatory bowel disease. Veterinaria 2017;31:1–12.
29. Niina A, Kibe R, Suzuki R, et al. Improvement in Clinical Symptoms and Fecal Microbiome After Fecal Microbiota Transplantation in a Dog with Inflammatory Bowel Disease. Vet Med Res Rep 2019;Volume 10:197–201.
30. Furmanski S, Mor T. First Case Report of Fecal Microbiota Transplantation in a Cat in Israel. Isr J Vet Med 2017;72:35–41.
31. Rojas CA, Entrolezo Z, Jarett JK, et al. Microbiome Responses to Fecal Microbiota Transplantation in Cats with Chronic Digestive Issues. Vet Sci 2023;10:561.
32. Linnehan BK, Kodera SM, Allard SM, et al. Evaluation of the safety and efficacy of fecal microbiota transplantations in bottlenose dolphins (Tursiops truncatus) using metagenomic sequencing. J Appl Microbiol 2024;135:lxae026.
33. Rowland IR, Mallett AK, Wise A. The Effect of Diet on the Mammalian Gut Flora and Its Metabolic Activities. CRC Crit Rev Toxicol 1985;16:31–103.

34. Tang-Wing C, Mohanty I, Bryant M, et al. Impact of diet change on the gut microbiome of common marmosets (Callithrix jacchus). Wilkins LGE, ed. mSystems 2024:e00108-24.

35. Williams CL, Caraballo-Rodríguez AM, Allaband C, et al. Wildlife-microbiome interactions and disease: exploring opportunities for disease mitigation across ecological scales. Drug Discov Today Dis Models 2018;28:105–115.

36. Williams CL, Ybarra AR, Meredith AN, et al. Gut Microbiota and Phytoestrogen-Associated Infertility in Southern White Rhinoceros. Dearing D, McFall-Ngai MJ, eds. mBio 2019;10:e00311-19.

37. Corsato Alvarenga I, Jackson MI, Jewell DE, et al. A Low to Medium-Shear Extruded Kibble with Greater Resistant Starch Increased Fecal Oligosaccharides, Butyric Acid, and Other Saccharolytic Fermentation By-Products in Dogs. Microorganisms 2021;9:2293.

38. Jackson MI, Waldy C, Cochrane C, et al. Consumption of identically formulated foods extruded under low and high shear force reveals that microbiome redox ratios accompany canine immunoglobulin A production. J Anim Physiol Anim Nutr 2020;104:1551–1567.

39. Mohanty I, Allaband C, Mannochio-Russo H, et al. The changing metabolic landscape of bile acids – keys to metabolism and immune regulation. Nat Rev Gastroenterol Hepatol 2024. Available at: https://www.nature.com/articles/s41575-024-00914-3. Accessed June 1, 2024.