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# The Gastrointestinal Microbiome: A Review

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The gastrointestinal microbiome is a diverse consortium of bacteria, archaea, fungi, protozoa, and viruses that inhabit the gut of all mammals. Studies in humans and other mammals have implicated the microbiome in a range of physiologic processes that are vital to host health including energy homeostasis, metabolism, gut epithelial health, immunologic activity, and neurobehavioral development. The microbial genome confers metabolic capabilities exceeding those of the host organism alone, making the gut microbiome an active participant in host physiology. Recent advances in DNA sequencing technology and computational biology have revolutionized the field of microbiomics, permitting mechanistic evaluation of the relationships between an animal and its microbial symbionts. Changes in the gastrointestinal microbiome are associated with diseases in humans and animals including inflammatory bowel disease, asthma, obesity, metabolic syndrome, cardiovascular disease, immune-mediated conditions, and neurodevelopmental conditions such as autism spectrum disorder. While there remains a paucity of data regarding the intestinal microbiome in small animals, recent studies have helped to characterize its role in host animal health and associated disease states. This review is intended to familiarize small animal veterinarians with recent advances in the field of microbiomics and to prime them for a future in which diagnostic tests and therapies will incorporate these developments into clinical practice.

**Key words:** Metagenomics; Microbiomics; Microbiota; Prebiotics; Probiotics.

All mammals are inhabited by communities of microorganisms essential to the normal form and function of the host. In terms of cellular composition, genetic diversity, and metabolic capacity, the host animal should be regarded as a multispecies hybrid organism composed of host and microbial cells operating in dynamic and symbiotic equilibrium. The diverse consortium of bacteria, archaea, fungi, protozoa, viruses, and their collective genome found on and within the body comprises the microbiome.[1,2] The microbiome makes vital contributions to energy homeostasis, metabolism, gut epithelial health, immunologic activity, and neurodevelopment.[1–3] The microbiome is dynamic and subject to important changes during the life of the host in response to a variety of factors including diet, environment, medical interventions, and disease states.

Microbiomics is an emerging investigative field seeking to identify the constituents of the microbiome, analyze the microbial genome, characterize the interactions between the microbiome and host, and determine its influence on the pathobiology of disease (Fig 1). The advent of high-throughput DNA sequencing technologies, coupled to advances in bioinformatics, has revolutionized the field of microbiomics. Commonly, these methods involve amplification and sequencing of targeted microbial DNA regions followed by statistical analysis of microbial identity and diversity based on sequence similarity and comparisons to reference microbial genomic databases. For bacteria, these methods target the 16S ribosome gene (16S rDNA), a conserved bacterial gene region with hypervariable sequences that differ between species. More recently, investigators have employed whole-genome shotgun sequencing which permits taxonomic analysis, investigation of microbial gene repertoires, and identification of nonbacterial microbes that are excluded from 16S rDNA sequencing. Differences in sample processing techniques, sequencing technologies, and statistical methods complicate any direct comparison between different sequencing studies. However, these methods have supplanted culture-based approaches, permitting the analysis of increasingly complex characteristics of the microbiome. Most studies of

**Abbreviations:**

| | |
|---|---|
| 16S rDNA | 16S ribosomal subunit gene |
| FISH | fluorescence in situ hybridization |
| FMT | fecal microbial transplant |
| FOS | fructooligosaccharide |
| GALT | gut-associated lymphoid tissue |
| IBD | inflammatory bowel disease |
| LPS | lipopolysaccharide |
| MAMP | microbe-associated molecular patterns |
| NOD | nucleotide-binding oligomerization domains |
| PCR | polymerase chain reaction |
| PRR | pattern recognition receptor |
| RCDI | recurrent *Clostridium difficile* infection |
| SCFA | short-chain fatty acids |
| SIBO | small intestinal bacterial overgrowth |
| TLR | toll-like receptors |
| TRD | tylosin-responsive diarrhea |
| $T_{reg}$ | T regulatory cell |

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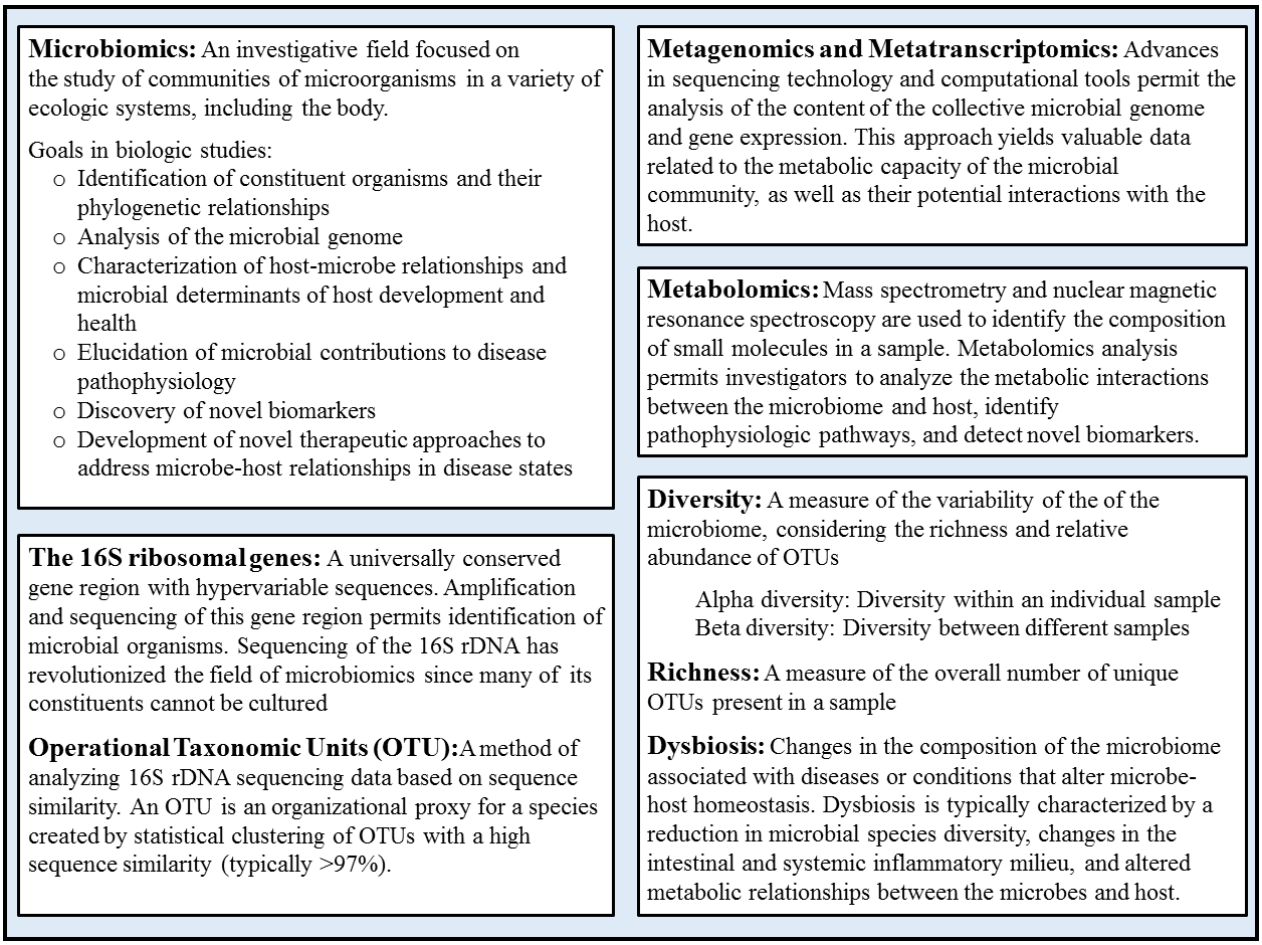


**Fig 1.** Definitions and methods of analysis of the intestinal microbiome. Terminology and technical aspects related to the analysis of microbiomic data can be a barrier to understanding the available literature. The information here is not intended to be a thorough technical overview of the field. Rather, these definitions are meant to familiarize the reader with the fundamental concepts and terminology present in the literature.

the gastrointestinal microbiome in mammals have focused on the fecal microbiome, although it is unclear how well fecal samples reflect the microbiomes of other intestinal regions. One study in humans demonstrated a strong positive association between fecal and mucosa-associated microbial communities for some genera (e.g, *Bifidobacteria spp*.), while other studies have revealed relevant differences between the microbial populations in feces and mucosal biopsy samples.[4–6]

To date, there are limited comprehensive reviews of the intestinal microbiome that integrate recent developments from studies in humans into the existing literature regarding the importance of the intestinal microbiome in dogs and cats. Notably absent in existing reviews is a discussion of the acquisition of the neonatal microbiome and its impact on host health and development. Given the importance of the microbiome in normal host physiology and its role in association with a variety of diseases, a thorough review of this rapidly evolving topic is warranted. This review is intended to update the veterinary community on recent advances in knowledge from studies in humans and other mammals, and to summarize the current state of microbiomics in small animal medicine, with an emphasis on the bacterial inhabitants of the gastrointestinal tract.

## Assembly and Development of the Intestinal Microbiome

Before birth, all mammals were thought to be sterile with inoculation with microbes occurring at the time of birth. There is some evidence supporting the vertical transfer of microbes before birth, but these findings are controversial and more research will be required to verify these claims.[7–9] Most studies in human neonates suggest that the early colonizers of the infant intestine are acquired through contact with maternal and environmental microbes.[10,11] This concept is supported by studies demonstrating an association between the composition of the infant intestinal microbiome and the route of delivery in humans. Infants delivered vaginally harbor microbial communities, dominated by *Lactobacillus spp*. and *Bifidobacterium spp*., which are similar to their mother's vaginal canal.[11,12] Conversely, infants delivered by cesarean section are colonized by microbial communities composed of common skin microbes with

*Staphylococcus* as the predominant genus.[11] These studies suggest that neonatal acquisition of the intestinal microbiome is dependent on the organisms encountered in the first hours and days of life from the mother and environment. These early colonizers are enriched for genes governing the metabolism of sugars found in milk as well as those involved in the de novo synthesis of folate, representing critical metabolic functions in the developing host gut.[13,14] Partial restoration of the microbiome was accomplished in a pilot study of newborns delivered via C section that were exposed to the maternal vaginal fluids at birth.[15] The gut, oral, and skin microbiomes in these infants were enriched with bacterial communities that resembled vaginally delivered babies.[15] These, and other studies in humans, show that the early stages of colonization are characterized by profound interindividual and temporal variation and limited intra-individual taxonomic diversity in the composition of the intestinal microbiome.[10,14,16–18]

In the third trimester of pregnancy, the human maternal intestinal microbiome undergoes a profound shift resulting in decreased compositional diversity with associated systemic inflammatory and metabolic consequences resembling metabolic syndrome.[19] Interestingly, neonates do not tend to inherit these microbial signatures from their mothers. Instead, the neonatal gut microbiome more closely resembles that of its mother in the first trimester. This is evidence for the presence of a selective environment in the neonatal gut, permitting colonization by certain organisms while inhibiting others. In mice and humans, the formation of a microbial-mucosal symbiotic unit is guided by complex interactions between bacterial and host immunologic signaling pathways that result in immunologic tolerance and niche colonization.[20,21] One study in human infants found that the mode of birth, antibiotics, and diet influenced the pace of microbial colonization, but not the pattern of maturation.[22] These studies provide evidence that microbial colonization of the neonatal gut is initially influenced by the source and quality of the postparturient inoculum, but then follows a developmental maturation process guided by complex, reciprocal interactions between the host and microbiome.

Once the intestinal microbiome is established, an orderly developmental program unfolds during which its composition and functional capacity converge toward a mature configuration.[10,13,18,23,24] This process is punctuated by dramatic shifts that correlate with events and circumstances in early childhood, especially the introduction of solid food during weaning.[10,13,22,25] These factors effect a transition whereby the gut microbiome evolves the ability to scavenge energy from complex carbohydrates, metabolize xenobiotics, and participate in vitamin biosynthesis (cobalamin, biotin, thiamine, among others); all features of a mature microbiome.[26–28] Interestingly, bacterial communities involved with plant polysaccharide metabolism might be present before the introduction of solid food, preparing the infant gut for plant-derived nutrients.[14,18,29] This finding emphasizes the impact of the microbiome on the developmental trajectory of the host. The end state of this process is characterized by high interpersonal variation and is influenced by diet, geography, and culture. However, the developmental succession of the microbiome toward increasing compositional and functional diversity and complexity is conserved across diet and geography.[13]

In addition to the microbiome's role in the adaptation to complex nutrients, it is also involved in a variety of other critical developmental processes. Recent studies in laboratory animals point toward a window in which the intestinal microbiome directs the development of the immune system, gut epithelium, and brain, among other body systems.[30–34] While compositional and functional changes in the intestinal microbiome are associated with normal developmental milestones, negative health events in childhood also have important impacts on the gut microbiome. Maternal health status and milk quality, antibiotic administration, premature birth, and malnutrition are associated with abnormal developmental patterns in the intestinal microbiome.[32,35–39] Malnutrition for instance has been associated with profound dysbiosis leading to persistent gut microbial immaturity that is refractory to nutritional treatment.[38,39] Additionally, asthma, atopy, childhood obesity, and autism spectrum disorder have all been linked to childhood antibiotic use in humans.[32,40] In 1 study, antibiotic administration before 6 months of age was associated with the development of asthma in children with no family history of asthma.[41] Despite the evidence that abnormal development of the intestinal microbiome has long-term implications on host health, the causal contributions of these abnormalities to disease states have yet to be elucidated.

Little is known about the acquisition and development of the intestinal microbiome in dogs and cats. Knowledge of the topic is limited to a handful of studies in kittens and puppies. To date, only 1 longitudinal study of developing kittens has been published.[42] The results of this study confirmed that, as in humans, the early fecal microbiome is characterized by a high degree of interindividual variation and that intra-individual diversity and compositional stability increase with age. Also similar to humans, the relative abundance of *Lactobacillus* and *Bifidobacterium* decreased with age, while *Bacteriodes* and bacterial genes associated with the ability to metabolize complex carbon sources increased with age. However, there was no major change in taxonomy or bacterial gene repertoires between weeks 30 and 42 in these kittens. Another study found that there was little change in the fecal microbiome of kittens between 8 and 16 weeks of age.[43] These results suggest that, as in humans, the developing gut microbiome converges upon a temporally stable configuration as kittens mature. Only 1 study has employed DNA sequencing methods to investigate the gut microbiome in puppies, revealing that temporal instability and substantial interindividual variability are also features of the puppy fecal microbiome.[44]

Other studies designed to assess the impact of diet have revealed valuable information about the intestinal microbiome in young cats. In 1 such study, the fecal microbiomes of kittens fed high- or moderate-protein diets after weaning were more similar between

littermates at 8 weeks of age, but at 12 weeks of age, the effect of kinship was diminished and diet emerged as the primary factor driving interindividual similarities between the cats.[45] This is evidence that the composition of the neonatal intestinal microbiome is strongly influenced by environment and kinship, whereas subsequent developmental shifts are driven by diet. These investigators also identified changes in the composition of the gut microbiome after weaning, a finding that is confirmed in several other studies of young cats, which also demonstrate age-related changes in the microbial gene pool.[43,45–47] Interestingly, one of these studies found associations between diet-induced changes in the microbiota and complex metabolic and hormonal effects in the host.[46] In these kittens, fecal presence of *Lactobacillaceae* was negatively correlated with body weight and positively correlated with blood leptin concentrations. *Bifidobacteraceae* was positively correlated with blood ghrelin and negatively correlated with blood triglycerides. Kittens fed a high-protein, low-carbohydrate diet had a lower relative abundance of these organisms. The association between diet-induced compositional differences in the fecal microbiome and hormones regulating satiety and lipid metabolism is intriguing and is suggestive of a large role for the intestinal microbiome in regulating developmental metabolism. However, the specific macronutrients, micronutrients, or both, responsible for these changes, and the mechanisms driving these host-microbe interactions are unknown. Collectively, these findings are consistent with data in humans and might indicate a similar age-related convergence of the developing microbiome toward an increasingly diverse and more stable mature configuration. It is also increasingly clear that the intestinal microbiome is an active participant in host developmental physiology.

Studies in humans and small animals suggest that the patterned maturation of the gut microbiome is a conserved feature of mammalian development. The findings summarized here are suggestive of a critical developmental window, during which long-term host developmental trajectories are set in association with age, environment, illness, and diet-related changes in the gastrointestinal microbiome. However, there is a paucity of longitudinal studies of the developing microbiome in small animals and fundamental questions regarding its maturation, and impact on host physiology, remain unanswered. Additional longitudinal studies are needed to characterize the origin, structure, and functional characteristics of the neonatal gut microbiome in veterinary patients. The compositional and temporal patterns by which these features evolve toward a mature configuration are unknown. The long-term impact of events that alter maturation of the microbiome in small animals during the developmental window is also undescribed and deserves further investigation.

## Maintenance and Stability of the Gastrointestinal Microbiome in Dogs and Cats

In contrast to the infant, the mature human intestinal microbiome is characterized by profound compositional and functional diversity, and long-term temporal stability.[48] These essential features of the adult human microbiome appear to be mirrored in adult dogs and cats. A variety of factors influence the gut microbiome's composition as well as its long-term stability and resilience to change (Fig 2). Several studies describe the

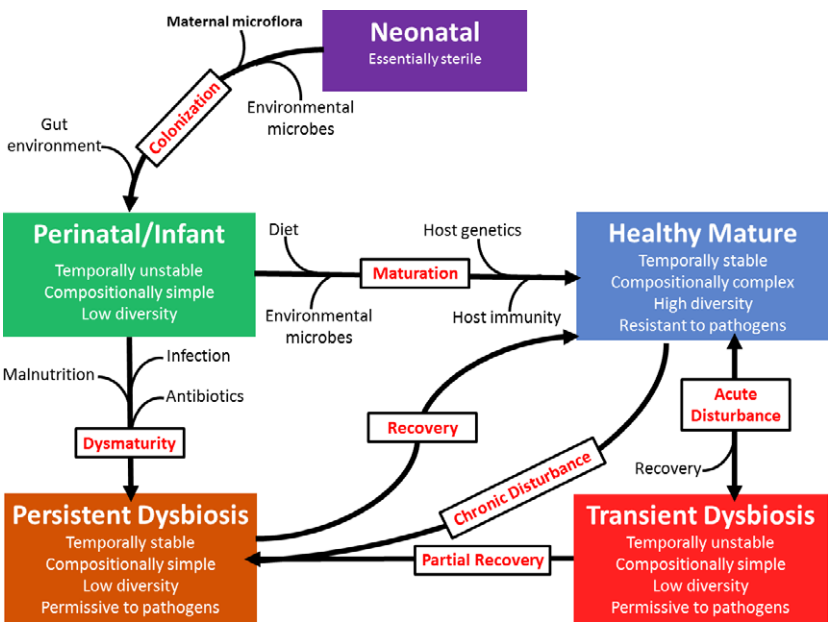


**Fig 2.** Factors influencing gut microbial development and steady states over time. The adult gastrointestinal microbiome is remarkably stable, although several factors influence gut microbial steady states starting at the time of birth. The infant microbiome is derived from maternal and environmental organisms and develops under selective pressure in the gut. Diet, drug administration, and disease states impact the intestinal microbiome, leading to transient or persistent dysbiosis, depending on the nature of the insult and its duration.

gastrointestinal microbiome in healthy, mature dogs and cats. Additionally, there are reports describing the impact of heredity, diet, and medical interventions on its compositional structure, diversity, and functional capacity. The predominant bacterial phyla in the intestinal tracts of adult dogs and cats are Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, and Actinobacteria (Fig 3). The relative abundances of these phyla vary between the existing published reports, likely a result of differences in individual host genetics, diet, age, sample collection method and source, DNA extraction techniques, and analytic methodology. As in humans, the majority of studies in dogs reveal that the predominant bacterial phyla in the canine feces are Firmicutes and Bacteroidetes.[26,49–52] *Clostridium* is the dominant genus with a relative abundance ranging from 7–22%.[51,52] In contrast with humans and other animals, Fusobacteria appears to be relatively abundant in canine feces, and some studies have found that Fusobacteria was either the most abundant phylum, or that it was codominant with Firmicutes and Bacteroidetes.[53–55] The relevance of the increased proportion of Fusobacteria in dogs relative to humans is unclear, although members of the phylum have been associated with inflammatory bowel disease (IBD) and colorectal cancer in humans.[56,57] Conversely, dogs with IBD tend to have lower relative abundances of Fusobacteria, compared with healthy dogs.[58] Although cats are obligate carnivores with no strict requirement for complex carbohydrates, their colonic microbiome is similar in composition to dogs and other mammals. Most studies indicate that Firmicutes are the predominant phylum in cats with a relative abundance ranging from 36 to 92%.[52,59–61] Within Firmicutes, *Clostridiales* is the most abundant order and *Clostridium* the most abundant genus.[52,59,60] In contrast with dogs, Proteobacteria comprise a substantial proportion of the feline colonic microbiome, with a relative abundance of 7.9–14%.[59–61]

Although most studies in dogs and cats have focused on the characterization of the fecal microbiome, there are substantial differences in the composition of the microbiome in different segments of the gut in dogs and cats.[59,62] In dogs, *Lactobacillus* is distributed uniformly

| Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|
| Firmicutes | *Clostridia* | *Clostridiales* | *Clostridiaceae* | *Clostridium* |
| | | | *Ruminococcaceae* | *Ruminococcus* |
| | | | | *Faecalibacterium* |
| | | | *Eubacteraceae* | *Eubacterium* |
| | *Bacilli* | *Lactobacilliales* | *Lactobacillaceae* | *Lactobacillus* |
| | | | *Streptococcaceae* | *Streptococcus* |
| | | | | *Enterococcus* |
| | *Erysiphelotrichia* | *Erisophelotrichales* | *Erysiphelotrichaceae* | *Turicibacter* |
| | | | | *Catenibacterium* |
| | | | | *Coprobacillus* |
| | | | | *Allobaculum* |
| | *Negativicutes* | *Selenomonadales* | *Selenomonadaceae* | *Megamonas* |
| | | *Veillonellales* | *Veillonellaceae* | *Dialister* |
| | | | | *Megasphaera* |
| | | | | *Veillonella* |
| Bacteroidetes | *Bacteroidia* | *Bacteroidales* | *Prevotellaceae* | *Prevotella* |
| | | | *Bacteroidaceae* | *Bacteroides* |
| Actinobacteria | *Coriobacteriia* | *Coriobacteriales* | *Coriobacteriaceae* | *Collinsella* |
| | | | *Atopobiaceae* | *Olsenella* |
| | | *Eggerthellales* | *Eggerthellaceae* | *Slackia* |
| | | | | *Eggerthella* |
| | *Actinobacteria* | *Bifidobacteriales* | *Bifidobacteriaceae* | *Bifidobacterium* |
| Fusobacteria | *Fusobacteriia* | *Fusobacteriales* | *Fusobacteriaceae* | *Fusobacterium* |
| Proteobacteria | *Gammaproteobacteria* | *Enterobacteriales* | *Enterobacteraceae* | *Escherichia* |
| | | | | *Shigella* |
| | | *Aeromonadales* | *Succinivibrionaceae* | *Succinivibrio* |
| | | | | *Anaerobiospirillum* |

**Fig 3.** Taxonomy and phylogeny of common constituents of the gastrointestinal microbiome. Microbiomic studies rely on taxonomic classification of bacteria based on DNA sequencing analysis. This literature commonly features results in which organisms with similar names but different phylogenetic levels are listed side-by-side. This figure was prepared to provide readers with a reference containing the most common organisms found in the gut, along with their phylogenetic relationships. Taxonomic and phylogenetic information courtesy of the NCBI Taxonomy Browser (https://www.ncbi.nlm.nih.gov).

throughout the intestinal tract whereas the relative abundances of *Enterobacteriales, Fusobacteriales*, and *Clostridiales* varied along its length. *Enterobacteriales* had a higher relative abundance in the canine small intestine, and *Clostridiales* was the predominant order in the duodenum and jejunum (40% and 39%, respectively).[62] This contrasts with the ileum and colon in which *Fusobacteriales* and *Bacteroidales* were the predominant bacterial orders. In cats, *Lactobacillales* is also distributed along the length of the gut, particularly in the jejunum and colon.[59] The feline small intestinal microbiome is predominated by Firmicutes and Bacteroides, whereas Proteobacteria and Actinobacteria are the dominant phyla of the ileum, and the colonic microbiome contains a higher abundance of Firmicutes, Proteobacteria, and Fusobacteria.[59]

The influence of host genetics on the composition of the gut microbiome is unclear. Some 16S rDNA sequencing studies suggest that the intestinal microbiome of related humans is more similar in composition than that of unrelated individuals and that specific host gene elements are associated with the heritability of the gut microbiome.[63–65] Similarly, the fecal microbiomes of genetically related dogs are more similar to each other than unrelated dogs.[55] However, a study of several hundred humans, including mono and dizygotic twins, found that the gut microbiome of related individuals was no more similar than that of unrelated individuals within the same geographic and cultural region.[13] This study, and others like it, reveals that humans from different geographic regions can be distinguished from one another based on the composition and genomic features of the intestinal microbiome.[13,48] These studies implicate diet and environment as the primary influences on long-term steady states of intestinal microbial communities. Indeed, recent studies of humans and other mammalian species found that diet was the primary influence on the structural and functional characteristics of the fecal microbiome.[66–68] Likewise, diet affects both the short-term composition and long-term composition in the canine and feline fecal microbiome.[26,43,46,49,50,54,61,69–75]

The convergence of the intestinal microbiome around a stable, mature configuration has led to the emergence of the concept of a phylogenetic "core" microbiome.[76] However, recent studies in dogs and humans refute the existence of a "core" microbiome. Metagenomic investigations have revealed that despite phylogenetic diversity, there is substantial interpersonal overlap in functional bacterial gene families.[13,65] These findings suggest that the "core" microbiome is defined by the contents of the collective bacterial genome rather than a conserved phylogeny. Interestingly, 1 study in dogs revealed that the relative abundance of *Prevotella* and *Bacteroides* is inversely proportional to that of Fusobacteria, a relationship that is also present in humans.[55] Considering the broad functional overlap in microbial gene pools between individuals, the profound compositional variation in the intestinal microbiome of mammals likely precludes the existence of a "core" microbiome centered on a conserved phylogeny. It is more likely that gut microbial niches are occupied not by specific organisms, but by organisms with overlapping functional repertoires and that the "core" microbiome is defined by a relatively constant microbial gene pool despite variation in compositional structure.

Analysis of the metagenome of the canine intestinal microbiome has revealed functional similarity to mice and humans.[50] These data show that the predominant bacterial gene categories in the canine gut include carbohydrate metabolism (12–13% of all sequences), protein and amino acid metabolism (8–9 and 7%, respectively), cell wall synthesis (7–8%), vitamin and cofactor synthesis (6%), and nucleic acid synthesis (7%).[50] The feline gut microbiome is also involved in carbohydrate and protein metabolism, cell wall biosynthesis, nucleic acid synthesis, and cofactor and vitamin biosynthesis.[77] Genes for virulence and antibiotic resistance are also common features of human, canine, and feline microbial gene pools.[50,77,78] Interestingly, functional gene families do not differ between dogs fed high- and low-fiber diets or between healthy dogs and those with intestinal dysbiosis.[50,79,80] However, it is likely that current shotgun sequencing and statistical methods are not yet sensitive enough to detect small but potentially important changes in the metagenome under experimental and disease conditions.[79] Future studies of the fecal transcriptome by quantitative PCR might permit the detection of biologically relevant changes in microbial gene expression from sequencing methods that are broader in nature.

In addition to diet and environment, medical interventions influence steady states in gut microbial communities. For instance, the human fecal microbiome is profoundly affected by the administration of ciprofloxacin which reduces taxonomic diversity and richness.[81,82] After discontinuation of the medication, the microbiome only partially recovers to its pre-antibiotic configuration resulting in a new, long-term compositional steady state that differs from the pre-antibiotic state. In dogs, macrolide administration induces compositional shifts in the fecal microbiome characterized by a decrease in compositional diversity and richness.[83] In this study, the response to tylosin administration varied between individual dogs, although treatment resulted in a persistent reduction in bacterial species diversity and richness in 40% of dogs. In addition to antibiotics, other medications can also affect bacterial populations in the canine gastrointestinal tract. The administration of a proton pump inhibitor (omeprazole) to dogs causes a reduction in *Helicobacter* and an increase in the proportion of Firmicutes and Fusobacteria in the stomach of dogs.[84] Additionally, omeprazole administration was associated with an increase in the duodenal bacteria as well as changes in the fecal microbiome characterized by an increased relative abundance of *Lactobacillus* and a decrease in proportions of *Faecalibacterium* and *Bacteroides*.[84]

The studies reviewed here suggest that the adult intestinal microbiome of humans, dogs, and cats is characterized by long-term stability and profound compositional and functional diversity. It is also clear that vital host physiologic processes are governed by

interactions between the host and resident microbiome and that conditions which affect the structure of the microbiome may impact host health. The long-term stability and susceptibility to change during pathologic and aberrant metabolic states make the gut microbiome a viable diagnostic and therapeutic target. The following sections of this review will explore host-microbiome relationships in more detail, while reviewing diseases associated with alterations in the intestinal microbiome.

## Microbe-Host Interactions and Associations between Dysbiosis and Disease States

The ancient maxim, "All disease begins in the gut," apparently stated by Hippocrates in the 3rd Century B.C.E, has, in principle, held up to modern scientific scrutiny. The microbiome participates in vital physiologic and immunologic processes including energy homeostasis and metabolism, the synthesis of vitamins and other nutrients, endocrine signaling, prevention of enteropathogen colonization, regulation of immune function, and metabolism of xenobiotic compounds.[27,28] Indeed, many gastrointestinal and systemic diseases have been associated with aberrant gut microbial communities. It is unclear whether the microbiome participates directly in the pathogenesis of these disease states. However, a growing body of evidence implicates the microbiome directly in pathogeneses of disease via complex interactions between the microbiome and host metabolic and immune systems. Here, we will review the current state of knowledge regarding the role of the intestinal microbiome in conditions affecting gastrointestinal and systemic health, with an emphasis on obesity and chronic enteropathies.

Interactions between the microbiome and host depend on local crosstalk networks with each intestinal niche harboring its own microbial community, adapted and responsive to the local environment. These communities differ along the length and width of the GI tract as well as within the different layers of mucus. The ileum and colon are much more active immunologically than the proximal small intestine.[85] The architecture of the host-cell-microbe interaction is extremely complex and the interacting cells differ considerably. The microbiome in small intestinal crypts, for instance, regulates enterocyte proliferation by influencing DNA replication and gene expression, while the microbiome at the tips of the villi regulate the expression of genes involved in metabolic and immune function.[34] Enterocytes and Paneth cells in turn regulate luminal bacteria through the production of mucus and antibacterial factors.[86]

The maintenance of mucosal immunologic homeostasis is an enormous task demanding discrimination between billions of harmless microbes and a rare, pathogenic invader. Both innate and adaptive immune responses function to prevent pathogen colonization and direct local and systemic inflammatory responses in the face of continuous exposure to foreign microbial and dietary antigens. These defense mechanisms consist of redundant systems to guard against rogue pathogens. The gut-associated lymphoid tissue (GALT) provides the bulk of immune surveillance and defense. Lymphoid follicles containing T cells, B cells, and dendritic cells are poised to initiate either an inflammatory or an anti-inflammatory response depending on the specific microbial signals. Specialized epithelial cells (M cells) sample luminal antigens and deliver them to dendritic cells for assessment.[87] Innate lymphoid cells might also be important in the maintenance of intestinal barrier integrity and the development of tolerance to commensals.[88] Additionally, microbes are essential in the development of GALT as evidenced by severely underdeveloped lymphoid follicles, Peyer's patches, and mesenteric lymph nodes in germ-free animals.[89] Colonization of germ-free mice with segmented filamentous bacteria stimulates development of the host's immune system; these mice have relatively normal numbers of Th1, Th17, and $T_{reg}$ cells compared with conventional mice.[90]

Recognition of microbes begins with the 2 main pattern recognition receptor systems (PRRs): Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain molecules (NODs).[91] These are widely expressed in and on intestinal epithelial cells, as well as macrophages, and dendritic cells in the intestine. These PRRs recognize molecular patterns, called microbe-associated molecular patterns (MAMPs), on pathogens and commensals.[91] Once a microbe has been recognized, internalized, or has invaded the epithelial layer, it initiates an immunologic response appropriate for the microbe (Fig 4). Microbes can exert both pathogenic and protective effects depending on specific microbial signaling through PRRs and MAMPs and the subsequent immune response. Protective effects are mediated by the downregulation of pro-inflammatory cytokines (IL-8, IL-12, IL-23) and upregulation of anti-inflammatory cytokines such as IL-10 produced by T regulatory ($T_{reg}$) cells.[92–94] In the case of commensals, the dendritic cells present the antigen to naive T cells, which differentiate into $T_{reg}$ cells. Secretion of anti-inflammatory cytokines ensues, propagating systemic and local tolerance.[95,96] Commensal organisms also decrease the migration of phagocytes, which traffic microbial antigens to local lymphoid tissues and promote B- and T-cell activation.[97] Additionally, goblet cell differentiation and production of the protective mucosal mucus layer are stimulated by commensal organisms.[98] Conversely, pathogenic bacteria cause dendritic cells to secrete pro-inflammatory cytokines that cause naïve T cells to differentiate into Th1 and Th17 cells leading to pro-inflammatory immune responses.[95–97] Additionally, TLRs 4 and 5, which are normally present on the basolateral side of epithelial cells, are expressed on the apical side in states of chronic inflammation, promoting a pro-inflammatory steady state.[22] Different gram-negative bacteria have LPS modifications that vary in their potential to stimulate TLR so that not all bacteria will induce the same immunologic response.[99]

While there is an intuitive link between the microbiome and local gut inflammation, recent metabolomic studies reveal that the microbiome is also a crucial participant in host systemic metabolism. Metabolomics is the study of small molecules present in biologic

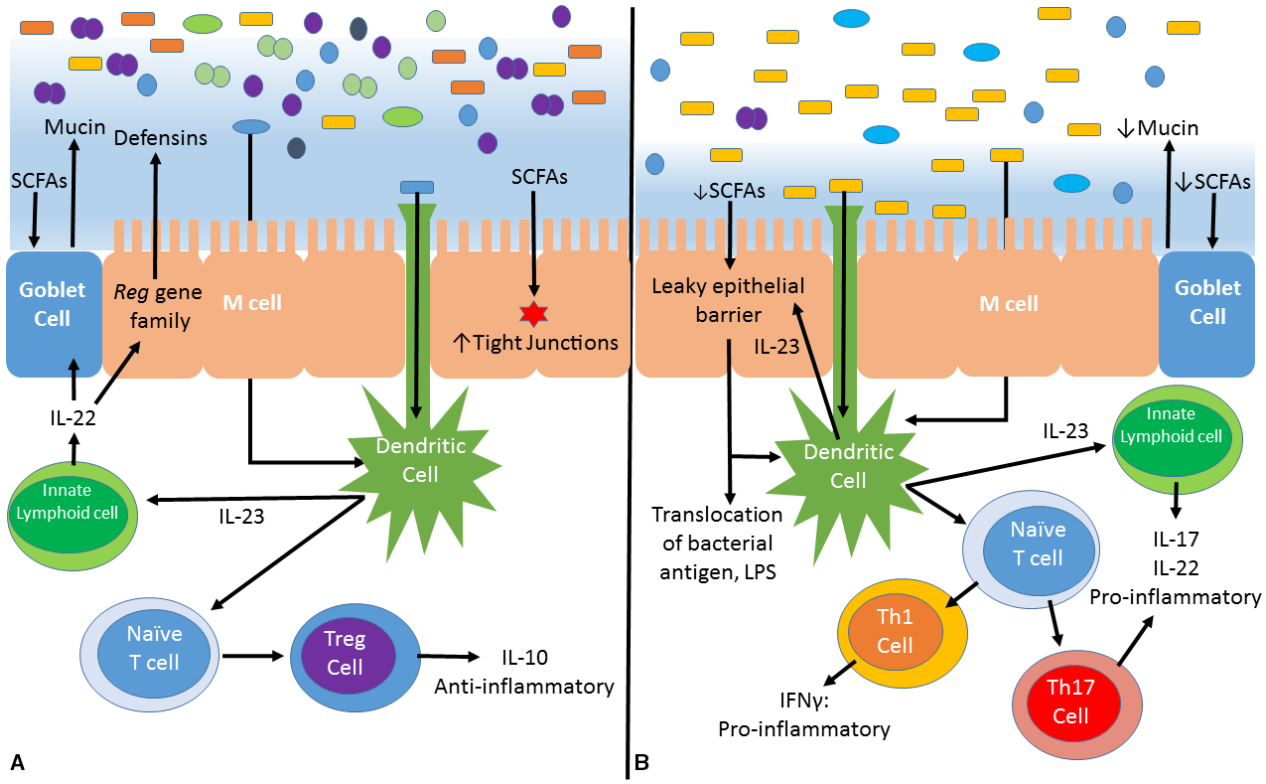


**Fig 4.** Balance between anti and pro-inflammatory states in the intestinal mucosa. Mucosal-microbial homeostasis is a complex and rapidly evolving subject. This simplified diagram is intended to provide examples of some of the most well-described immunologic interactions between the microbiota and host mucosa. This image represents a schematic cross section of the gut epithelium, mucus layer, and lumen. The colorful rods, ovals, and circles at the top of the image represent luminal microbiota and the blue haze in which some reside is the mucus layer. A. In healthy individuals, pro- and anti-inflammatory signals are balanced such that commensal organisms are recognized and tolerated, while pathogens are prevented from penetrating the mucus layer and underlying epithelium. SCFAs strengthen epithelial tight junctions and stimulate the production of the mucus layer. Commensal organisms are recognized by dendritic cells and specialized epithelial M cells, promoting the maturation of naïve T-lymphocytes into $T_{reg}$ cells that secrete anti-inflammatory and immunomodulatory cytokines. Innate lymphocytes stimulate the overlying epithelial cells to secrete antimicrobial defensins. B. States of dysbiosis are characterized by reduced diversity in the resident microbiota depicted here as an increased relative abundance of yellow rods. Recognition of a shifting antigenic milieu by dendritic cells and M cells results in the maturation of naïve T cells into Th1 and Th17 cells, both of which secrete pro-inflammatory cytokines. Additionally, altered microbial metabolism (e.g, reduced SCFA production) promotes degradation of host protective factors, such as the mucus layer, that stabilize gut microbial communities and prevent colonization by pathogens.

specimens whereas the metabolome is the collective pool of metabolites in an organism or individual sample. A recent study in mice reported significant differences in the plasma metabolomes of germ-free and conventional mice, concluding that the microbiome interacted with approximately 10% of host metabolic pathways and that numerous plasma metabolites are derived exclusively from the microbiome.[100] In 1 study, dogs with acute diarrhea had concurrent fecal dysbiosis and an altered systemic metabolic state, providing additional evidence for the metabolic links between microbe and host.[80] Another study revealed changes in the fecal microbiomes and serum metabolomes of dogs with IBD compared to healthy controls.[101] Interestingly, the IBD dogs improved clinically after treatment but this was not accompanied by changes in the microbiome or serum metabolite profiles.

In addition to their contribution to systemic metabolic states, microbial metabolites also modulate mucosal immune responses. Microbial fermentation of complex carbohydrates (e.g, resistant starch, inulin) produces short-chain fatty acids (SCFA), including acetate, propionate, and butyrate. SCFAs are an essential energy source for colonocytes, maintain the epithelial barrier by strengthening tight junctions, help to regulate intestinal motility, and stimulate the production of anti-inflammatory compounds.[39] SCFAs also bind a G protein receptor on neutrophils to decrease migration, leading to downregulation of inflammation and have been shown to limit colitis in experimental animal models.[39,102–104] Alternatively, diminished SCFA production, the elaboration of toxic microbial metabolites, interruption in mucosal barrier function, and microbe-mediated host immune dysregulation perpetuate the conditions which promote the establishment of a new long-term, pro-inflammatory steady state.[94,105] One study of dogs with acute diarrhea identified reductions in fecal propionate that was correlated with a decrease in fecal *Faecalibacterium*.[80] Other classes of metabolites, such as bile acids have immunomodulatory

effects.[106–108] Primary bile acids are metabolized by gut microbes, generating secondary bile acids. Secondary bile acids bind and activate several host receptors to a greater extent than the primary bile acids of the host.[109]

Diversity of the intestinal microbiome is emerging as a critical determinant of host health, and a loss of diversity has been associated with a variety of gastrointestinal and systemic diseases in humans and other mammals.[110–115] Intestinal dysbiosis is a loosely defined concept referring to any change in the intestinal microbiome that adversely affects the health of the host organism. Dysbiosis is characterized by broad shifts in community microbial compositional structures, reduced species diversity, and changes in the relative proportion of particular organisms, whereby symbionts normally representing a small proportion of the microbiome develop pathogenic features.[94,113] Although the nature of dysbiosis varies according to the individual, as well as the pathologic condition, reductions in the relative proportion of obligate anaerobes and increases in facultative anaerobes including pathogens such as *E. coli, Salmonella, Proteus, Klebsiella,* and *Shigella* are common features of dysbiosis in humans and laboratory animals.[105] Dysbiosis does not always involve pathogens as absence of important commensals can be detrimental without the presence of pathogens.

Dysbiosis and mucosal inflammation are interrelated and a plethora of data points to inflammation as either a cause of dysbiosis, a consequence of it, or some combination of the 2. Research in murine and rat models of IBD suggests the microbiome is essential to the initiation and progression of mucosal inflammation, which is absent or attenuated in these animals when raised in germ-free conditions.[116–120] In humans, many of the genes that are associated with IBD are involved in the regulation of host-microbe interactions.[121] For instance, variation in genes (NOD2 and ATG16L1) involved with the intracellular processing of bacterial components has been associated with Crohn's disease in humans.[121] Additionally, research in animal models has demonstrated that transfer of the microbiome from animals with IBD to healthy animals increased the susceptibility of the healthy animal to the development of IBD.[16,122] Dysbiosis is a consistent feature of IBD in humans and, while the clinical characteristics of IBD differ between humans and animals, researchers have identified states of dysbiosis in dogs and cats with IBD and other gastrointestinal disorders.[58,74,123]

Dysbiosis in dogs is also characterized by decreased diversity and a reduction in the relative proportion of species that are known to produce SCFAs.[58,74,113] Historically, the term small intestinal bacterial overgrowth (SIBO) had been used to describe quantitative and qualitative changes in the intestinal microbiome, based on cultures of duodenal and jejunal juice, associated with exocrine pancreatic insufficiency and antibiotic-responsive enteropathies in dogs.[124] More recently, the term dysbiosis has been used to describe alterations in the gut microbiome associated with disease states or other conditions (e.g., antibiotic administration) in order to reflect the more discriminating findings of modern genomic studies. Several microbial community compositional abnormalities have been identified in dogs with various chronic and acute enteropathies.[72,74,125–127] It is clear from these data that intestinal dysbiosis is a common feature of a variety of enteropathies in dogs. The number of studies is small and, as in humans, no universal pattern of dysbiosis has emerged from the data. A majority of sequencing-based studies in dogs with dysbiosis have identified a lower relative abundance of Bacteroidetes in addition to increased proportions of *Clostridia* and Proteobacteria, as well as expansion of other organisms that are minor constituents of the microbiome of healthy animals.[58,101] There were substantial differences between samples from dogs with IBD and healthy control dogs, and dogs with IBD had significantly lower alpha diversity compared with healthy controls. However, there was no correlation with diet, body condition, or several other factors with the microbiome analysis.[58] A dysbiosis index created by these authors was negatively correlated with alpha diversity in this study.[58] Recently, a fecal qPCR panel provided rapid diagnosis of dysbiosis based on the dysbiosis index score.[a] In this study, a negative dysbiosis index score indicated eubiosis and as the value became greater, it indicated a stronger deviation from eubiosis such that a value of 0 had 84% sensitivity and 86% specificity for differentiation of dysbiosis from eubiosis. To date, no 16S rDNA or shotgun sequencing studies have been published in cats with intestinal dysbiosis; however, 2 studies employing fluorescence in situ hybridization (FISH) suggest that cats with chronic enteropathies have significant dysbiosis compared with healthy cats. One study identified decreased *Bifidobacterium spp* and *Bacteroides spp.* as well as increased *Desulfovibrio*, a producer of toxic sulfur-containing compounds, in the feces of cats with IBD.[123] Another FISH study identified increased mucosa-associated *Enterobacteriaceae* associated with inflammatory infiltrates in cats with IBD.[128] The clinical importance of these findings is unclear, particularly when attempting to compare small intestinal biopsies and fecal analysis. Future research efforts should be aimed at elucidating the pathophysiologic mechanisms underlying intestinal dysbiosis in veterinary patients with enteropathies.

There is particularly strong evidence for an etiologic link between obesity and metabolic syndrome and certain forms of intestinal dysbiosis. Obesity is a major problem in human and veterinary medicine, and diagnostic and therapeutic tools to prevent and treat obesity are in critical demand. Recent studies have revealed compelling associations between obesity and intestinal microbiome in humans, laboratory animals, and pets. Proposed mechanisms for these associations include increased dietary energy harvest, microbe-induced changes in host glucose and lipid metabolism, microbial signaling through host endocrine systems, and chronic low-grade inflammation leading to insulin resistance.[129] A recent metagenomics study found that the fecal microbiomes of obese humans are enriched for genes that govern the harvesting of dietary energy though the production of SCFAs.[110] SCFAs are a direct energy

source but are also effective signaling molecules, acting through receptors (GPR41) in enteroendocrine cells to decrease gut transit time, increase SCFA recovery, and promote adiposity.[130] One study conducted in germ-free mice inoculated with cecal contents from conventional mice suggests a direct, causal link between the intestinal microbiome and increased adiposity.[131] Weight gain in these mice occurred despite calorie restriction via increased intestinal monosaccharide absorption and increased hepatic lipogenesis. Furthermore, the microbiome in these mice suppressed a host gene (*Fiaf*) coding a circulating lipoprotein lipase inhibitor (Angptl4), causing an increase in triglyceride deposition in adipose tissue.[132] Lipopolysaccharide (LPS), a component of gram-negative bacterial cell walls, has been associated with low-grade systemic inflammation in mouse models of obesity. LPS levels are also positively correlated with total energy intake and are higher in patients with type 2 diabetes.[129] High-fat diets induce a reduction in the relative proportion of *Bifidobacteria*, resulting in increased intestinal permeability and higher plasma LPS levels.[133] Conversely, probiotic supplementation with *Bifidobacteria* lowers serum LPS levels, improves glucose tolerance, and reduces inflammation.[129]

Dysbiosis characterized by an increased Firmicutes: Bacteroidetes ratio has been identified in obese mice compared with lean mice as well as in high-fat diet-induced obese mice.[65,110,111] Recent 16S rDNA sequencing studies of fecal samples from obese humans, including 1 study of 154 mothers and twins with obese and lean phenotypes, reveal that human obesity is also associated with decreased diversity and lower proportions of fecal Bacteroidete*s* and that weight loss is associated with a proportional increase in Bacteroidetes.[110,111,134,135] Similarly, obese cats have lower proportions of Bacteroidetes than healthy cats, a feature of moderately and severely obese cats, suggesting that changes in the microbiome occur before clinically relevant obesity is apparent.[136] A single study comparing lean and obese pet dogs did not identify large shifts in the microbiome associated with obesity.[127] However, this study did identify changes in the fecal microbiome under different feeding conditions in a research colony of beagles. In these dogs, *Clostridiales* increased with *ad lib* feeding while *Gammaproteobacteria* and *Alphaproteobacteria* decreased in dogs fed a calorie-restricted diet. These results should be interpreted with caution given the influence of the different environmental factors on the microbiomes of dogs from research colonies compared with pets. Additional studies will be required to clarify the role of the microbiome in obesity in dogs and cats.

In humans and laboratory animals, a diverse array of other systemic diseases including immune-mediated conditions (rheumatoid arthritis, atopy, and asthma) and neurodevelopmental disorders (autism spectrum disorder) have been linked to intestinal dysbiosis.[32,33] These associations have not been identified in small animals and are beyond the scope of this review. Despite the paucity of data, and lack of clarity regarding the microbiome's role in the pathogenesis of the conditions reviewed here, there are clear links between the intestinal microbiome and systemic health. Therefore, the intestinal microbiome should be considered a viable diagnostic and therapeutic target in future studies designed to assess the clinical impact of these findings.

## Therapeutic Manipulation of the Gastrointestinal Microbiome

Therapeutic manipulation of the intestinal microbiome is generally achieved through modification of the diet, administration of prebiotics, probiotics, or antibiotics, and more recently, fecal microbiome transplantation (FMT).[b,c,d] The therapeutic targets of these interventions and their efficacy are not well established and have only recently been described in the literature. These therapies are meant to shift microbial community steady states associated with dysbiosis to those associated with health. Several studies have reported amelioration of clinical signs after treatment with various microbial concoctions, as will be discussed in this section. Interpretation of these findings is challenging as changes in the microbiome might not necessarily translate into clinical improvements, clinical improvements can occur without detectable changes in the microbiome, and clinical signs might be unaffected, or transiently affected by alterations in the microbiome.

### *Prebiotics*

Although fewer studies exist, the potential benefits of prebiotic supplementation, especially by the addition of plant-derived polysaccharides, is a mainstay of treatment directed at modification of the intestinal microbiome. Referred to as prebiotics, these include inulin, nonstarch polysaccharides found in some cereal grains and seaweed or algae, disaccharides (lactulose), and polysaccharides including fructooligosaccharides (FOS) and galactooligosaccharides. Prebiotics are fermented by colonic bacteria, generating end-products such as SCFAs that provide essential nutrients for the enteric epithelium. They also induce anti-inflammatory $T_{regs}$, and lower luminal pH.[137–139] Induction of $T_{regs}$ maintains an anti-inflammatory milieu and the acidic pH prevents specific pathogens from gaining a foothold. Prebiotics are also speculated to protect the gut epithelium by increasing the mucus layer, elongating the microvilli, increasing numbers of epithelial cells, and by preventing adherence of pathogenic strains to the epithelial cells.[140,141] Other SCFAs such as propionate appear to induce de novo generation of $T_{regs}$ in the peripheral immune system.[142] A newly published study in mice revealed that when deprived of fiber, commensal bacteria will degrade the protective mucosal mucus layer, permitting invasion by commensals and pathogens alike.[143]

Several studies have evaluated the effects of prebiotics in dogs. In 1 study, chicory root, a source of inulin, improved fecal scores, increased *Bifidobacteria*, and decreased *C. perfringens* in the feces of healthy dogs.[144] Meta-analysis of 15 studies including 65 different treatment conditions showed that fecal SCFAs

concentrations increase linearly with prebiotic dose.[145] This analysis also revealed that fecal *Bifidobacteria* and *Lactobacillus* increase with prebiotic dose, although no changes were observed for pathogenic *C. perfingens* or *E. coli*. The impacts of prebiotics were not related to the composition of the dog's diet, suggesting that prebiotic therapies can provide benefits independent of diet. The potential benefits of prebiotic supplementation are also evident in cats. In 1 study, pectin administration increased levels of *Lactobacilli* and fecal SCFA concentrations, while supplementation with FOS led to increased *Bifidobacteria* and decreased *E. coli* as well as increased concentrations of fecal butyrate.[146]

### *Probiotics*

Probiotics are formulations of live organisms that confer beneficial effects on the recipient when delivered in adequate amounts. Proposed mechanisms through which probiotics improve host health include reducing intestinal permeability by upregulation of tight junction proteins, increasing mucin secretion by goblet cells, increasing secretion of defensins which prevent pathogen colonization, production of SCFAs, stimulation of IgA secretion, decreasing luminal pH, and enhancing and directing immune cells to promote tolerance to commensals while maintaining protection against pathogens.[147] Even nonviable organisms might confer health benefits by adhering to the mucus layer and stimulating immune function in dogs.[148,149] Capsular polysaccharide from *Bacteroides fragilis*, for instance, activates $T_{regs}$ and decreases severity of colitis in mice.[150] The most commonly used probiotic organisms include *Lactobacillus* and *Bifidobacterium*. Additional bacteria used include *Bacillus* and *Streptococcus* as well as the yeast, *Saccharomyces boulardii*. It is essential to remember that probiotic organisms are often strain specific.[b] In order to remain viable, probiotics must survive the acidic environment of the stomach and bile acid to colonize the intestines. One study detected probiotic species in 11 of 12 dogs during probiotic administration but was not able to detect the probiotic species before or after completion of the trial.[151] However, probiotics appear to confer benefits without changing the microbiome permanently and transient colonization is still associated with beneficial effects in the host.[152]

Evidence of a therapeutic effect of probiotics dates to the early 20th century. A study describing improvement in autoimmune arthritis after supplementation with live cultures of *Streptococcus lacticus* and *Bacillus bulgaricus* was reported in 1909.[153] More recent studies have shown that *Bifidobacterium longum* subspecies *longum* (JCM 1217) has been shown to protect against *E. coli* O157:H7-induced enteropathogenic infection in mice.[154] A large body of research has focused on a probiotic mixture of lyophilized bacteria representing *Lactobacillus* (*L. casei, L. plantarum, L. acidophilus, L. bulgaricus*), *Bifidobacterium* (*B. longum, B. breve, B. infantis*), and *Streptococcus salivaris* termed VSL#3. In a meta-analysis of VSL#3 in humans with ulcerative colitis, the supplement was associated with longer remission times compared with placebos.[155] In 29 children with ulcerative colitis, VSL#3 was associated with higher remission rates (92.8%) compared with placebo (36.4%) as well as decreased relapse (21.4%) compared with placebo (73.3%) at 1 year.[156] A systematic review of 23 randomized controlled trials (RCTs) in 1917 patients reported that probiotics were associated with shortened duration of acute gastroenteritis compared with placebo in humans.[157] Another review of 3 RCTs revealed improvement in clinical signs associated with rotavirus diarrhea in the probiotic group compared with a placebo in infants.[158] In yet another meta-analysis including 2963 patients, the probiotic *Lactobacillus rhamnosus* was associated with decreased duration of diarrhea compared with a placebo.[159] A systematic review of probiotics for prevention of antibiotic-associated diarrhea reported that probiotics were associated with a reduction in diarrhea in humans receiving antibiotics.[160]

Probiotic supplementation benefited small animals in several clinical trials. A small clinical trial with a probiotic strain of *Saccharomyces boulardii* improved clinical signs in dogs with IBD and protein losing enteropathy.[a] In dogs with food-responsive diarrhea treated with lyophilized *Lactobacillus* for 21 days along with diet change, there were increased *Lactobacilli* and decreased *Enterobacteria* in the feces accompanied by improved clinical signs.[161] In another study of 36 dogs with acute gastroenteritis, a probiotic combination improved clinical signs compared to a placebo.[162] *Enterococcus faecium* SF68 is a lactic acid-producing strain that has been shown to increase antibody secretion in mice with Giardiasis as well as decrease fecal shedding of Giardia.[163] When administered to puppies, *E. faecium* SF68 was associated with increased serum IgA levels.[163] In a shelter-based study, this probiotic, administered with metronidazole, improved fecal scores compared to dogs treated with metronidazole alone.[164] In kittens from a shelter, *E. faecium* SF68 significantly decreased the incidence of diarrhea.[165] Only 9.5% of the probiotic group developed diarrhea compared with 60% of kittens that were not given probiotics. There was also a decrease in fecal *C. perfringens* and an increase in *Bifidobacteria* associated with probiotic administration. In another large trial of 217 shelter cats, *E. faecium* decreased episodes of diarrhea compared with a placebo.[166] A multispecies synbiotic containing *Enterococcus faecium, Bacillus coagulans,* and *Lactobacillus acidophilus*, along with prebiotics, improved fecal scores and reduced the incidence of diarrhea in healthy Alaskan sled dogs.[167] In dogs with IBD, VSL#3 improved clinical signs and increased immunehistochemical staining for FoxP3+ cells, a marker for $T_{regs}$, compared with a control group that was treated with metronidazole and prednisolone.[168]

### *Antibiotic Administration*

Therapeutic manipulation of the microbiome can also be achieved through the administration of select antibiotics. In several human studies, metronidazole was associated with disease remission in patients with Crohn's disease.[169–171] Metronidazole is a nitroimidazole

antibiotic and antiprotozoal that is commonly used in small animals with acute and chronic gastrointestinal disorders. A recent study in healthy dogs revealed that metronidazole decreases fecal bacterial diversity but increases the proportions of putatively beneficial bacteria such as *Bifidobacterium*.[172] Tylosin is a macrolide antibiotic that is typically used to treat chronic enteropathies in dogs, especially a variant of antibiotic-responsive diarrhea termed tylosin-responsive diarrhea (TRD). One study evaluating its effect in healthy dogs revealed that it causes a reduction in jejunal bacterial diversity and increased the proportion of putatively beneficial *Enterococcus* species.[83] Interestingly, it also caused an increase in potentially pathogenic bacteria such as *C. perfingens, E. coli,* and *Pasteurella*, although no dogs in this study developed any new clinical signs of gastrointestinal disease during treatment. Clinical signs in dogs with TRD resolve within the first 3 days of therapy. Additionally, tylosin administration is associated with increases in *Enterococcus* species in dogs with TRD.[173] Rifaximin, a semisynthetic rifamycin with broad-spectrum activity which undergoes minimal systemic absorption, was found to significantly improve clinical signs in a cohort of dogs with IBD, suggesting potential manipulation of either pathogenic strains (decreased), beneficial commensals (increased), or a combination of the 2.[174,175]

### *Fecal Microbiome Transplantation (FMT)*

Fecal microbiome transplantation entails the transfer of feces from a healthy donor into the intestinal tract of a diseased recipient. The first modern record of FMT, for treatment of humans with pseudomembranous colitis, was reported in 1958 and all 4 patients that were treated survived.[176] The route of delivery varies between studies and includes oral capsules, nasogastric, nasoduodenal and colonoscopic administration, and via enema.[177] It is unclear whether the benefits of FMT are derived from the transfer of viable commensals, or via the delivery of viruses, proteins, bile acids, vitamins, SCFAs, and the myriad of other substances not yet identified in feces, or some combination of these. The first condition where the FDA has granted investigational new drug status for FMT in the United States was *Clostridium difficile* infection. Patients with recurrent *C. difficile* (RCDI) have decreased proportions of fecal Bacteroidetes and Firmicutes, and an ~90% cure rate is expected after FMT.[178,179] In patients with RCDI after FMT, the recipient's fecal microbiome mirrors that of the donor, up to 24 weeks after FMT.[180–182] There are also reports of clinical improvement after FMT in humans with IBD, multiple sclerosis, myoclonus dystonia, and refractory ulcerative colitis.[23,183] A double-blind, randomized controlled trial of FMT in 18 men with metabolic syndrome used autologous feces as the control and donor feces from lean men for the treatment arm.[184] The treatment group had improvement in insulin sensitivity and increases in butyrate-producing bacteria after 6 weeks of weekly infusion compared with the control group.

Two recent abstracts provide information from case reports of FMT in dogs. One dog with eosinophilic IBD improved substantially after an FMT enema, and remained free of clinical signs for 3 months afterward.[c] Sequencing of 16S rDNA revealed that 2 days after FMT, the dog's fecal microbiome was more similar to the donor that its own pretreatment sample. Additionally, the post-FMT fecal sample had higher microbial diversity than the pre-FMT sample. Another report documented successful FMT in 8 dogs with refractory *C. perfingens*-associated diarrhea.[d] In this report, FMT was also administered by enema and diarrhea resolved in all dogs after treatment. These results, as well as those from studies in humans, indicate that FMT is a highly successful treatment for Clostridium-associated diarrhea in humans and dogs, although the efficacy in the treatment for other gastrointestinal diseases such as IBD and systemic diseases associated with dysbiosis is unclear. Although reports suggest that FMT is a safe therapeutic approach, some humans with ulcerative colitis developed a fever and increased C-reactive protein after FMT.[185] Given the paucity of data demonstrating its safety and efficacy, indications for its use, and uncertainty in regard to selection of appropriate donors, preparation of the donor fecal samples, and delivery methods, the authors do not recommend FMT except for cases of *Clostridium*-associated diarrhea and other chronic enteropathies that are refractory to standard therapies. A recent commentary provides a thorough review of FMT in veterinary medicine.[186]

## Conclusion: Unanswered Questions and Future Directions for Research

The gastrointestinal microbiome is an essential contributor to mammalian health that participates in vital physiologic processes and guides host development. Abnormalities in gut microbial populations have been associated with a variety of gastrointestinal and systemic diseases, making the intestinal microbiome a viable diagnostic and therapeutic target. While recent advances in DNA sequencing and computational technology have revolutionized the field of microbiomics, many fundamental questions remain unanswered. Future directions for research should aim to elucidate the mechanisms underlying interactions between the microbiome and host, describe the process of microbiome maturation during host development and its impact on early-life and adult health outcomes, clarify its role in the pathogenesis of disease states, and assess the viability of diagnostic tests and therapies designed to assess and treat conditions associated with intestinal dysbiosis.

## Footnotes

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## Supporting Information

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