When mammals are born, their gastrointestinal (GI) tract is sterile.¹ Within the first 24 hours of life, microbes begin to populate the GI tract. The resulting microbial population is called the GI microbiota. Evolution has led to a stable, mutually beneficial relationship between the host and these microbes.¹ This relationship is complex, and we are just beginning to understand the GI microbiota and how it influences health and disease.
The microbiota is composed of living microorganisms, including bacteria, fungi, protozoa, viruses, parasites, and archaea (single-celled organisms known as prokaryotes). The GI tract of domestic animals is home to several hundred different genera of bacteria with >1000 phylotypes. These organisms outnumber the host’s own cells by a factor of 100, totaling $10^{10}$ to $10^{14}$ (10 with 10 to 14 zeros after it!). Likewise, some genetic components (e.g., DNA, mitochondria) of the microbiota exceed the host’s by 100 times or more. The collective genome of the microbiota is called the microbiome or, sometimes, the second genome.

**COMPOSITION**

The microbiota is not confined to the GI tract. Every part of the body that communicates with the external world has its own microbiota, the composition of which can vary between and within various sites. For example, the skin microbiota is different from the respiratory tract microbiota, and the composition of the skin or respiratory microbiota can differ between the upper and lower respiratory tract and between the aural and the inguinal skin.

The development of a healthy GI microbiota is critical to normal development and maintenance of health in the host, and an absence of the microbiota is incompatible with life; however, the GI microbiota changes throughout the life of an animal, depending on factors such as species, environment, and health. Dietary changes can also affect the composition of the GI microbiota, with relatively small changes in dietary fiber causing significant and detectable changes in the microbiota (see Effects of Diet on the Microbiota).

Production of metabolites by the resident microbiota is a driving force behind coevolution of the GI microbiota with the host. Because of this, the microbiota in any individual depends on the animal’s diet, environment, stage of life, disease state, and myriad other factors.

The GI microbiota is one of the densest microbial populations on the planet, and alterations to it have been associated with various diseases for some time. Its composition is viewed as a moving target, with some general aspects being understood, but details remaining unclear.

**Bacteria**

Historically, the assessment of the bacterial microbiota relied heavily on bacterial culture. This method of identification has some serious limitations because a large percentage of the microbiota consists of bacteria that do not grow under conventional culture conditions, leading to an underestimation of the true bacterial numbers and diversity of species. Culture is also limited in that it is impractical for detection of a single species in billions of bacteria.

Molecular methods for bacterial identification continue to evolve, allowing assessment of complex microbial populations and generating massive amounts of data. DNA gene sequencing through polymerase chain reaction allows better assessment of the true bacterial populations in the GI tract, but it is still only available through large research labs.

Oxygen requirement is one of the biggest determinants of the microbiota. The differing oxygen levels present in the stomach, small intestine, and colon lead to different bacterial populations in each area. Aerobic bacteria (i.e., bacteria that require oxygen for growth) are part of the microbiota of small intestine, while the large intestine is populated almost exclusively by anaerobic or facultative anaerobic bacteria (i.e., bacteria that do not grow well in the presence of oxygen).

The normal canine stomach typically contains high numbers of *Helicobacter* bacteria colonizing the superficial mucosa, gastric glands, and parietal cells. The colon also contains high numbers of mucosally associated bacteria, while the healthy small and large intestine have very few bacteria in the mucosa.

The primary bacteria found in the intestinal tract of dogs and cats are *Firmicutes* (~40%), *Bacteroides* (~30%), and *Fusobacteria* species (~30%). Specific beneficial bacterial species found in the largest amounts in dogs include *Enterococcus* (Enterococcus faecium and Enterococcus faecalis) and lactic acid species. Beneficial species in cats include *Lactobacillus*, *Enterococcus*, and *Bifidobacterium* species.

**Other Components**

Although bacteria account for the bulk of the microbiota, archaea, viruses, fungi, and parasites are also present. The archaea most likely play a minor role and may be present simply from environmental exposure. The viral microbiota is likely vast, with a combination of bacteriophages and mammalian viruses. At present, there is no easy or cost-effective way to assess this population. There have been no studies of the fungal component of the microbiota.
A balanced microbial ecosystem is crucial to the host’s optimal health. Physiologically, the microbiota stimulates the immune system, helps in defense against invading pathogens, and provides nutritional benefits to the host (BOX 1).\(^6\)

**Immune System Stimulation**

The immune system consists of innate and adaptive mechanisms that protect the animal from environmental pathogens.\(^7\) The **innate immune system** functions independently of previous exposure to organisms and includes mechanical barriers such as the skin, mucus, and epithelial linings, as well as cellular components such as macrophages and neutrophils.\(^7\) The **adaptive (or acquired) immune system** consists primarily of B and T lymphocytes and relies on generation of immunologic memory to recognize specific invading agents after an initial exposure.\(^7\) This memory can be acquired through transfer of antibodies from colostrum, vaccination, or infection.\(^7\) The two systems are not entirely separate but act as a continuum with much overlap in response.

Establishing a resident microbiota early in life is crucial for developing oral tolerance to normal bacterial and food antigens to prevent the onset of inappropriate immune response to these.\(^4\) This is usually accomplished through intake of colostrum during the early hours of life and through exposure to the maternal microbiota during birth, nursing, and cleaning. Animals that are born via cesarean section are not inoculated with maternal vaginal bacteria and show slower than expected immune development.\(^5\)

Typically, constant exposure to the microbial and antigenic components of the GI microbiota causes no problems for the host. Immunologic intolerance of the microbiota (caused by a congenital or immune system problem) can contribute to myriad infectious and inflammatory conditions, including diseases caused by opportunistic pathogens, allergies, metabolic diseases, neoplasia, and obesity.\(^3\)

**Defense Against Pathogens**

The GI microbiota defends the host against undesirable and pathogenic organisms by competing with them for adhesion sites and nutrients, secreting antimicrobial substances (bacteriocins), altering gut pH, assisting with GI transit time, and producing vitamins and growth factors for intestinal cells.\(^2,4\) As such, it plays a crucial part in the intestinal barrier system.

**Digestion and Nutrients**

The nutritional benefits of the microbiota to the host include aiding in nutrient breakdown and energy release from food; providing nutritional metabolites for enterocytes in the form of the short-chain fatty acids (SCFAs) propionate, butyrate, and acetate; and metabolizing drugs and other xenobiotics that otherwise cannot be absorbed by the host.\(^2,3,5,6\)

The GI microbiota is also important in the normal development of physiologic gut structures, and in specific pathogen-free (germ-free) animals, mucosal architecture is altered. Changes to the microbiota may lead to altered intestinal barrier function, damage to the brush border and enterocytes, increased competition for nutrients and vitamins, and increased deconjugation of intestinal bile acids.\(^7\) Damage to the intestinal absorptive surfaces such as the brush border and enterocytes directly affects the amount of energy extracted by the host from consumed food. If the bacterial population is not ideal, the microbiota can compete with the body for vital nutrients, decreasing the amount available for the host. Some bacteria produce nutrients required by the host, such as cobalamin; if these bacteria are not present, these nutrients are not available for the host.

SCFAs produced by the microbiota provide energy directly to the intestinal cells, provide important growth factors for epithelial cells, help modulate the immune properties of the intestinal tract, inhibit pathogenic bacterial overgrowth through modulation of colon pH, and influence gastrointestinal motility.\(^4\)

**BOX 1**

**Benefits of Gastrointestinal Microbiota**\(^2,5,6\)

- Forms a defensive barrier against potential pathogenic organisms
- Aids in nutrient breakdown and energy release from the diet
- Provides nutritional metabolites for enterocytes
- Helps regulate the host immune system
- Metabolizes substances (eg, drugs) that the host cannot
SCFAs are produced primarily from dietary fibers, which are defined as the edible portion of plants that is resistant to digestion by the host animal and absorption in the small intestine. Dietary fibers can be classified based on their physical or chemical characteristics and according to their effects on the bowel microflora; however, the most important characteristic for intestinal bacteria is fermentability. These fibers may be nonfermentable, such as cellulose and lignin; moderately fermentable, such as beet pulp, tomato pomace, and pea fiber; or highly fermentable, such as guar gum and pectin. Dietary fibers that can undergo bacterial fermentation include polysaccharides (eg, resistant starches, pectin, inulin, guar gum) and oligosaccharides (eg, fructooligosaccharides [FOS]). Most dietary fibers are polysaccharides, meaning they are composed of long strands of carbohydrates. The fermentability of a polysaccharide depends on the complexity of the molecule.

Fermentation (complete or partial) is performed by the resident bacteria in the distal small intestine and large intestine. The degree to which each fiber is fermented and the by-products produced are influenced by the structure of the carbohydrate and the individual animal’s microbiota. Utilization of most fermentable fibers is never 100%, and most natural fiber sources contain a range of carbohydrates (monosaccharides, disaccharides, and polysaccharides) of varying fermentability.

Complete fermentation produces hydrogen, carbon dioxide and water; incomplete fermentation produces methane, acetone, propionate, and butyrate. These products create a selection advantage for the bacterial species adapted for their use; therefore, a healthy microbiota helps perpetuate an environment that is beneficial to itself.

Butyrate

Butyrate is an SCFA produced through bacterial fermentation. In most domestic species, including dogs, it is an energy source for colonocytes; in dogs, it is also used by enterocytes. Butyrate can also increase colonocyte proliferation, intestinal mucosal weight, water and electrolyte absorption, and brush border enzyme activity. These are all seen as positive effects from fermentable fibers in the diet.

Fibers such as FOS, inulin, and resistant starch can lead to a significant increase in the production of butyrate, while fibers found in citrus pectin, citrus pulp, beet pulp, and cellulose yield relatively low levels of butyrate. This difference in butyrate production is directly related to the carbon structure of the individual fibers.

Prebiotics

A fermentable dietary fiber that leads to a shift in the microbiota with a positive effect in the host animal is defined as a prebiotic. Positive effects include reduction in the mucosal adherence of pathogenic bacterial species, reduction in the numbers of pathogenic species, and immune modulation of the host. Certain fibers, such as FOS and inulin, can stimulate the growth and/or activity of intestinal bacteria such as Lactobacillus and Bifidobacterium species. It has been proposed that increasing the numbers of these nonpathogenic bacterial species may have the positive effects listed above.

Ideally, prebiotics would be incorporated in the diet either as functional fibers in the ingredients or through the addition of actual vegetable products. While fiber supplements can be added to a diet, digestion is increased when the fibers are incorporated into the diet during manufacturing. Care must be taken to add only enough fermentable fiber to benefit the microbiota; too much fiber can cause GI distress through gas production or impaired motility. Fibers that lead to a higher concentration of butyrate in the proximal colon may

The microbiota is not confined to the GI tract. Every part of the body that communicates with the external world has its own microbiota.
have more positive effects than less fermentable sources of fiber. Currently, insufficient information is available to make an informed therapeutic recommendation beyond the initial introduction of mixed fermentable fiber sources, and proceeding with trial and error may be best to see which source is most effective in a particular animal.

**EMERGING MARKERS OF DYSBIOSIS**

*Dysbiosis* refers to an imbalance in the microbiota of the animal. Maintaining a balance of the microbes is important for intestinal homeostasis, and changes in the microbiota may directly or indirectly influence the host’s metabolic pathways.

GI disease can be caused directly by invading pathogens, but also by dysbiosis caused by opportunistic resident bacteria or by altered communication between the innate immune system and the commensal microbes living in the intestine. Due to the complex interactions between intestinal absorption and microbial metabolism, the exact cause of changes in serum concentrations of serum metabolites is often unknown, but a better understanding of the physiologic pathways is helpful to potentially pinpoint specific diseases.

**Cobalamin and Folate**

Serum concentrations of cobalamin and folate are important, albeit indirect, markers for GI disease. The uptake of cobalamin (vitamin B₁₂) and folate (vitamin B₉) from the small intestine depends on several factors. Disorders that may affect serum cobalamin and/or folate concentrations include small intestinal inflammation, exocrine pancreatic insufficiency (EPI), and small intestinal bacterial overgrowth (SIBO).

Cobalamin absorption is complex and requires a functioning GI tract. Major disorders that interfere with cobalamin uptake are EPI, distal or diffuse small intestinal inflammatory disease, and excess bacterial utilization of cobalamin in bacterial dysbiosis. Decreased absorption ultimately leads to metabolic consequences on a cellular level.

Dietary folate is typically poorly absorbable. In dogs, the receptors responsible for folate uptake are located exclusively in the proximal small intestine. Therefore, increased serum folate concentrations can be seen with proliferation of folate-producing bacteria in proximal SIBO, but not in SIBO of the distal small intestine (ie, ileum) or large intestine. In contrast, diffuse inflammation in the proximal GI tract may lead to damage of folate receptors and decreased serum folate concentrations.

**Bile Acids**

Another important pathway that can be altered in GI disease is bile acid metabolism. Changes in intestinal bile acid metabolism have been implicated as an important factor in intestinal inflammation in human IBD patients as well as *Clostridium difficile* infection; so far, these changes have not been studied in veterinary medicine. Unconjugated bile acids are toxic to epithelial cells, which may result in increased intestinal permeability from cellular damage. Altered bile acid profiles can lead to changes in fat absorption from the small intestine. Altered serum unconjugated bile acids have also been associated with altered small intestinal microbiota in dogs.

**D-Lactate**

A recent study has shown an increased serum D-lactate concentration in cats with various GI diseases. D-lactate is not normally found in any appreciable quantities in serum from mammals. The increase in serum D-lactate in cats with GI disease is possibly due to disturbances in the intestinal microbiota and increased bacterial production of D-lactate. An increase in D-lactate levels has been shown to lead to neurologic signs in some cats.

**CONCLUSION**

Associations between the intestinal microbiota and health or disease must be interpreted for what they are: associations and not necessarily causation. Separating causation and association can be difficult, particularly when there may be different influences on the disease process and microbiota, including differences in diet.
As we learn more about the composition and function of the microbiota, we will also need to address ways to keep this population as healthy and happy as the host it inhabits. The microbiota plays an important role in the development, immune regulation, and maintenance of health for all animals.3

References

Glossary

**Prebiotic**: A type of moderately fermentable fiber that can be used as an energy source by resident intestinal bacteria

**Probiotic**: A classification of live bacteria that are believed to confer benefits on the host when consumed

**Saccharides**: carbohydrates, including sugars; may be made of single molecules (monosaccharides), such as lactose, sucrose, and fructose; small groups of molecules (oligosaccharides), such as fructooligosaccharides; or chains of molecules (polysaccharides), such as inulin

**Specific pathogen-free (germ-free) animal**: Animals raised under laboratory conditions to prevent colonization by many normal bacterial species and are therefore guaranteed to be free of specific pathogens that may interfere with an experiment

**Xenobiotic**: Chemical substance found within an organism that is not normally naturally produced by or expected to be present in that organism; also, substances that are present in much higher concentrations than are usual.