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CHAPTER 2

Gastrointestinal Microbiota

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The intestinal microbiota is the collection of all live microorganisms that inhabit the gastrointestinal (GI) tract. The word *microflora* is often used synonymously, but *microbiota* (from *bios*, Greek: life) is the technically correct term. The intestinal microbiota plays an important role in GI health and disease, yet our understanding of the composition, dynamics, and functionality of the intestinal ecosystem remains rudimentary. The total microbial load in the intestine is estimated to be 10^{12} to 10^{14} organisms, approximately 10 times the number of body cells. It is estimated that the intestine harbors several thousand bacterial strains.[1,2] This mutually interacting system comprising the host cells and the resident microbes is termed the *intestinal microbiome*. The microbiota can be influenced by exogenous factors such as diet and antibiotic administration, but it is usually resilient to these changes and returns rapidly to its pretreatment state. Therefore long-term treatment strategies for modulating the microbiota are necessary. New molecular tools have improved our understanding of microbial diversity in the intestine. Although the major phylogenetic lineages are similar, the microbiota differs substantially at the level of species and strain in each individual animal of the same species. Yet despite these differences, the metabolic end products in the intestine are very similar between individuals. New metagenomic approaches suggest the presence of a "core microbiome," where the function of the intestinal ecosystem is independent of the presence of specific bacterial species or strains. For better understanding of microbial–host interactions in health and disease, future work must focus on the intestinal microbiome as one entity, evaluating its phylogenetic composition as well as metabolic functions.

Methods for Characterization of the Intestinal Microbiota

Practical Considerations

Methods for characterization of the intestinal microbiota are based on cultivation techniques or molecular tools (Fig. 2-1). The selection of the best approach depends on the study problem (e.g., detection of specific pathogens in clinical specimens, or general characterization of the intestinal ecosystem), the cost, and the availability of technologies. Each method has strengths but also limitations (outlined below). For general ecologic surveys of microbial communities, molecular high-throughput sequencing techniques yield the most information as they allow in-depth identification of microorganisms. For screening of specific pathogens, culture techniques and species specific polymerase chain reaction (PCR) assays may be most useful. Both of these methods are sensitive to sample handling and processing. Detailed instructions for sample collection and shipping for each particular assay should be acquired before sample submission as many laboratories use their own in-house culture or PCR assays. In the case of molecular methods, there is usually no standardization of DNA extraction or PCR protocols among laboratories, and such factors impact on the sensitivity and specificity of the assays. Improper DNA extraction, especially from fecal samples, may result in the presence of residual PCR inhibitors that cause false negative results. Because of the high sensitivity of PCR assays (theoretically a single target copy can be amplified), any DNA contamination can lead to false-positive results. A laboratory should be chosen that has expertise in molecular analysis, and that has validated each assay in the target specimen.

Bacterial Culture

Traditional evaluation of the composition of the canine and feline intestinal microbiota has been obtained using culture techniques. Bacterial culture is useful for assessing the viability of organisms, determination of an active infection, and antibiotic susceptibility testing in clinical specimens. Individual isolates can be typed for epidemiologic surveys of specific strains and their virulence factors. Culture is also valuable for understanding the metabolic properties of individual microbes. The value of bacterial culture is greatest when a clinical sample is evaluated for the presence of specific pathogens (e.g., *Salmonella*, *Campylobacter jejuni*).

Several limitations are associated with culture methods, especially if they are used to survey for the presence of unknown microorganisms in intestinal samples. Bacterial culture underestimates total bacterial numbers in the intestine, as microscopic counts (especially when using fluorescent dyes) are typically higher than the total viable counts obtained from culture. Although the majority of intestinal bacteria cannot be cultured, this does not necessarily mean that they are uncultivable, but rather, that insufficient information is currently available about their optimal growth requirements. Furthermore, many microbes depend on mutualistic interactions with other bacteria and the host, hindering their successful isolation in vitro. While recent advances have increased the cultivable fraction,[3] it is estimated that less than 10% of intestinal bacteria can be cultured, and an even smaller fraction can be correctly classified. Therefore studies of the intestinal ecosystem may

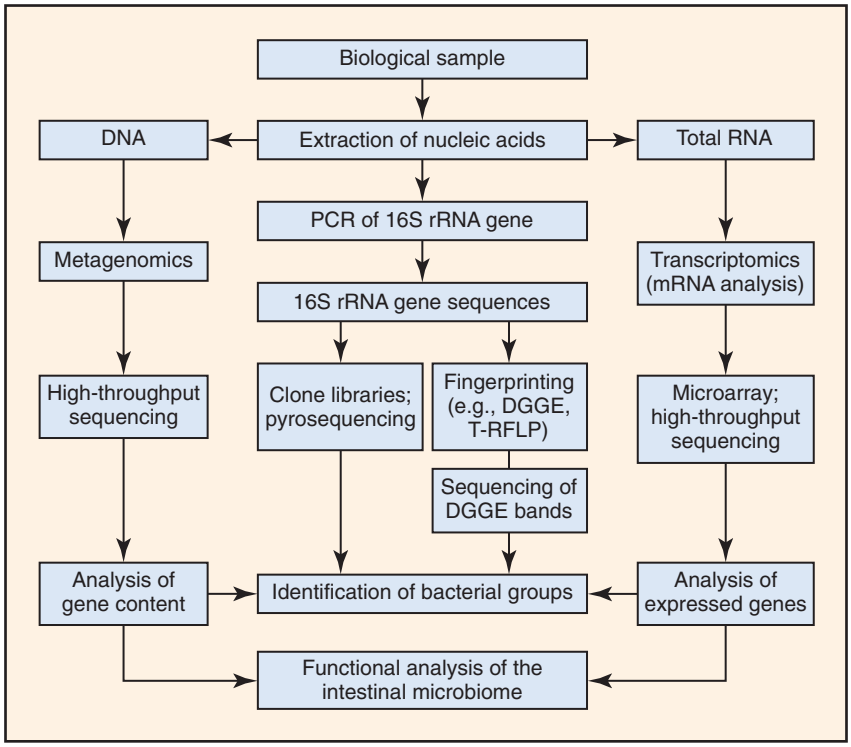

Figure 2-1 Molecular methods for characterization of the intestinal microbiome. PCR amplification of the 16S rRNA (ribosomal RNA) gene allows either direct identification of bacterial phylotypes or the creation of a molecular fingerprint representing the bacterial diversity in a sample. New metagenomic and transcriptomic approaches, based on high-throughput sequencing of DNA or messenger RNA (mRNA) without prior amplification of a specific gene, yield an overview over the gene content of the sample and therefore the functional properties of the intestinal microbiome.

exhibit bias toward the minor cultivable portion of the gut microbiota. The analytical sensitivity of bacterial culture depends on the organism and its growth requirements. Bacterial culture is associated with difficulties in handling, storing, and shipping of clinical specimens. Ideally, samples should be processed immediately to preserve anaerobic species. Many selective culture media lack sufficient specificity, and often organisms other than the target are enumerated.[4] Phenotypic and biochemical identification systems often fail to accurately classify many microorganisms, requiring DNA sequencing of isolates.

Molecular Techniques

Because bacterial culture underestimates microbial diversity, the use of molecular tools has now become the standard approach in microbial ecology.[5-7] The principle of these methods is that DNA or RNA is extracted from intestinal samples, and a specific gene is amplified with universal primers that target conserved regions (located up- and downstream of variable regions within the gene).[8] This approach allows in theory the amplification of DNA from all known and unknown bacterial species in a sample (see Fig. 2-1). The mixture of sequences can then be separated by subcloning and identified by sequencing, or they can be separated by methods that yield a "fingerprint" of the bacterial community.[8,9] The 16S ribosomal RNA (rRNA) gene is most commonly targeted as more than 1.6 million unique sequences are available in public databases (Ribosomal Database Project; http://rdp.cme.msu.edu/). Other more rarely used genomic targets include the 16S to 23S internal transcribed spacer (ITS) region or the chaperonin (cpn60) sequences.[10] If the sequence for a particular phylotype is known, specific PCR assays can be designed for its detection. Real-time PCR assays (with universal-, group-, or species-specific primers) can be used for quantitative analysis. Novel techniques analyze total genomic DNA or messenger RNA (mRNA) without prior amplification of specific genes and yield information about the gene content (metagenomics) or the expressed genes (transcriptomics) of the intestinal microbiome.

Molecular Fingerprinting

Molecular fingerprinting techniques are used for simultaneous analysis and comparison of microbial communities in multiple samples. These techniques provide information on microbial changes over time and in response to treatment. Available techniques include denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), and terminal restriction fragment length polymorphism (T-RFLP). The goal is to separate the mixture of PCR amplicons that were generated with broad range primers (universal or group specific) to yield a "fingerprint" of the bacterial community. This is achieved as each bacterial phylotype has a unique nucleotide composition (i.e., guanine+cytosine content). These differences in nucleotide composition result in unique melting behaviors of PCR amplicons. Each PCR amplicon reaches a specific melting point in a different position in a polyacrylamide gel, where it will denature and slow its migration. This banding pattern illustrates the bacterial diversity in the sample (Fig. 2-2). In DGGE, a gel containing a linear gradient of DNA denaturants is used, whereas in TGGE a temperature gradient is used for separation. Bands of interest can be excised and sequenced. DGGE and TGGE are inexpensive and rapidly performed. However, because DGGE/TGGE bands are usually short, only limited resolution of PCR amplicons can be achieved, and many bacterial phylotypes will have similar or

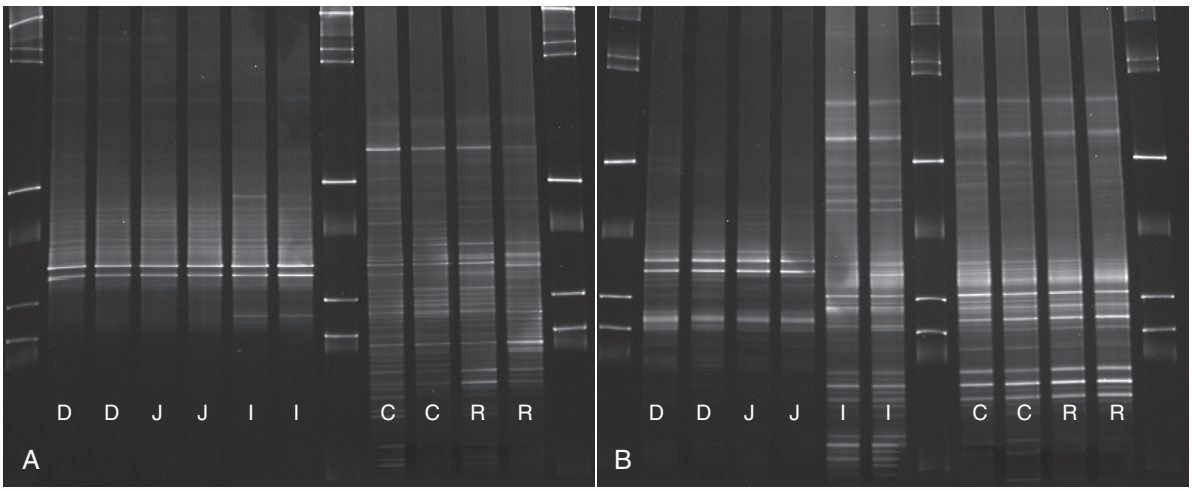

Figure 2-2 Denaturing gradient gel electrophoresis (DGGE) as an example for the use of molecular fingerprinting for profiling and comparison of microbial diversity between intestinal samples. This figure illustrates the differences in bacterial diversity in the various segments of the canine GI tract and the differences in bacterial communities between individual dogs (samples analyzed in duplicate). (C, colon; D, duodenum; I, ileum; J, jejunum; unlabeled lanes represent gel markers).

the same melting behaviors. Therefore, these techniques yield typically only 20 to 40 bands, capturing only changes in the predominant bacterial groups. The use of T-RFLP allows profiling, but also quantification of microbial communities. Bacteria are amplified in PCR assays containing a fluorescent labeled primer. The PCR products are then fragmented by size with sequence specific restriction enzymes. The fragments are separated by capillary electrophoresis with subsequent quantitative measurement of the fluorescence.

Identification of Bacterial Groups

The amplification of the 16S rRNA gene with universal primers that target conserved regions allows amplification of theoretically all bacteria present in the sample. For identification of individual bacterial phylotypes, PCR amplicons must be separated and sequenced. A commonly used method is the construction of 16S rRNA gene clone libraries.[6,7,10] The PCR amplicons are separated by ligation into plasmid vectors with subsequent transformation into *Escherichia coli* cells. These cells are plated on culture medium and grown overnight. Each cell forms a colony containing one plasmid with the original amplified 16S rRNA gene sequence. This plasmid can then be purified and sequenced. Although this approach is informative, it is laborious and not well suited for analysis of large sample numbers. Recently, new high-throughput sequencing platforms have been introduced that allow automated separation of PCR amplicons without the need for subcloning. These platforms (e.g., 454-pyrosequencing, Illumina) allow several thousand sequences to be analyzed within a few hours, yielding a deep coverage of the microbiota.[2,11] However, because of the high bacterial diversity in the intestine, groups of low abundance (especially pathogens of interest) may constitute such a low proportion of the total bacteria, that they still escape identification. Therefore, for the detection of particular groups of interest (i.e., *Bifidobacterium* spp.), the use of group specific PCR primers is recommended.

Techniques based on the 16S rRNA gene also have limitations. Bias is inherent during DNA extraction, primer selection, PCR amplification, and sequence analysis. Some commonly used primers and PCR protocols underestimate the presence of specific bacterial groups, especially those with a high guanine+cytosine content (e.g., *Bifidobacterium* spp.),[7] and some researchers use either a primer mix or group-specific primers for more accurate amplification.[11]

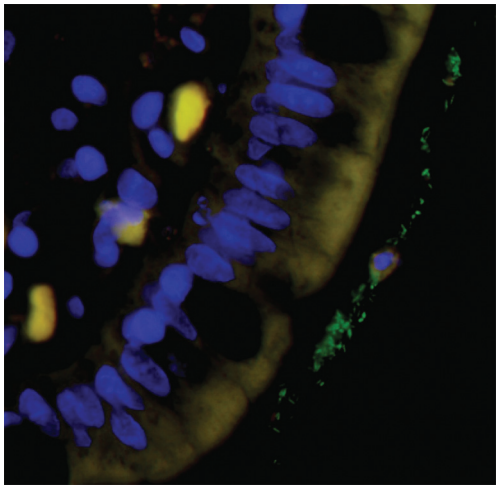

Figure 2-3 Fluorescent in-situ hybridization (FISH). The use of fluorescence-labeled FISH probes allows quantification and visualization of bacteria in relation to the epithelial mucosa (i.e., mucosa-adherent or invasive). This figure shows normal intestinal epithelium. Bacteria (*green*) are located in the mucus adherent to the epithelial cells. (Green, eubacterial probe labeled with 6-FAM; blue, nuclei of epithelial cells labeled with DAPI.) (Courtesy of Kenneth W. Simpson, Cornell University.)

Quantification of Bacterial Groups

Commonly used methods include quantitative real-time PCR assays,[10,12] fluorescent in-situ hybridization (FISH),[13] RNA dot blot hybridization,[14,15] and flow cytometry of fluorescent-labeled probes. Universal-, group-, or species-specific primers can be utilized. FISH allows for quantification of bacterial groups (Fig. 2-3), and this method also permits visualization of the location of bacteria in relation to the epithelium (i.e., intracellular, adherent, or invasive).

There is an inherent bias in use of the 16S rRNA gene for the purpose of absolute quantification. The 16S rRNA genes of bacteria

are organized into "operons" that vary in number from 1 to 15 among individual phylotypes. The operon number may also change during the growth phase and altered activity of cells.[16,17] Consequently, molecular results should be related to absolute cell counts with caution. It is more appropriate to express quantitative results as relative proportions to either total bacteria or to other bacterial groups.

Metagenomics and Transcriptomics

Analysis of 16S rRNA genes has provided new information about the phylogenetic diversity of the intestinal microbiota. However, to understand the impact of the microbiota on GI health it is necessary to (a) identify members of the intestinal ecosystem, and (b) explore the functionality of the microbial community. Metagenomics and transcriptomics are emerging fields in microbiology that are based on high-throughput sequencing techniques or the use of microarrays (see Fig. 2-1). In metagenomics, DNA extracted from a sample is sequenced without prior amplification of specific genes. This results in a snapshot of the gene pool and functional potential of the microbiome. For example, metagenomic approaches have revealed the existence of a "core microbiome" in the intestine, because despite obvious differences in bacterial composition between individuals, these individuals share common microbial genes and metabolic pathways.[18] In transcriptomics, mRNA is analyzed to provide a measure of gene expression within the intestinal microbiome. These techniques are expected to yield more in-depth understanding of microbial-host interactions in health and disease.

The Intestinal Ecosystem in Dogs and Cats

As a result of anatomical and physical differences, each intestinal compartment constitutes a unique ecosystem where microorganisms have their own niche and provide specialized functions by utilizing host nutrients and in return providing metabolites for host uptake (Table 2-1). Molecular studies reveal that each dog and cat has a unique microbial profile.[6,19] The microbiota is similar at higher phylogenetic level between individual animals of the same species, but it differs substantially at the level of species and strain, with typically only 5% to 20% overlap in bacterial species between individual animals. Bacterial counts and diversity increase along the GI tract and may vary between the intestinal lumen and the mucosa.[19,20] Bacterial counts vary between the fed and fasting state. The oral cavity is an important part of the intestinal ecosystem, because bacteria are constantly swallowed and they may be able to colonize parts of the intestine. The composition of the oral microbiota is complex. In one study, 84 different cultivable phylotypes were identified in the oral cavity of dogs, with the major groups being *Actinomyces*, *Porphyromonas*, *Fusobacterium*, *Neisseria*, and *Streptococcus* spp.[21] Oral bacterial counts can reach up to 10^7 colony-forming units per gram (CFU/g). The stomach harbors 10^1 to 10^6 CFU/g, while bacterial counts in the duodenum and jejunum of dogs and cats range from 10^2 to 10^9 CFU/g. This is considerably higher than found in the human duodenum ($<10^5$ CFU/g). Cats appear to have higher counts of anaerobic bacteria in the small intestine compared with dogs.[22] The ileum is a zone of transition between the small and large bowel and contains a more diverse microbiota and higher bacterial numbers (10^7 CFU/mL of contents) than the proximal small intestine. Colonic bacterial counts range between 10^9 and 10^{11} CFU/g of intestinal content. The predominant bacterial groups cultured from the canine and feline intestine include *Bacteroides*, *Clostridium*, *Lactobacillus*, *Bifidobacterium* spp., and Enterobacteriaceae (Table 2-2).

Because the vast majority of intestinal bacteria are not cultivable, molecular analysis (typically based on characterization of 16S rRNA) has expanded knowledge of diversity within the mammalian gut.[2,7] Several thousand individual phylotypes are estimated to inhabit the human colon.[1] There are approximately up to 900 bacterial phylotypes in the canine jejunum.[2] Despite this vast diversity, only 12 of the 55 known major phylogenetic lineages have been observed in the mammalian GI tract (see Table 2-2). The phyla *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Spirochaetes*, and *Fusobacteria* constitute almost 99% of all gut microbiota in dogs and cats. The remaining 1% is represented by the phyla *Tenericutes*, *Verrucomicrobia*, *TM7*, *Cyanobacteria*, *Chloroflexi*, and a few unclassified bacterial lineages. The relative proportions of these groups vary along the GI tract (see Table 2-2). Generally, proportions of aerobic bacteria or facultative anaerobic bacteria are higher in the proximal intestine, while anaerobes predominate in the colon. In the stomach, mucosa-adherent *Helicobacter* spp. predominate, followed by various lactic acid bacteria (e.g., *Lactobacillus* and *Streptococcus* spp.) and *Clostridium* spp. The proximal small intestine is more diverse than the stomach and harbors approximately 10 different bacterial phyla, with Clostridia, *Lactobacillales*, and *Proteobacteria* dominating.[2] *Proteobacteria* and *Spirochaetes* are present in higher proportion in the proximal GI tract and typically represent <1% of sequences in the large intestine of healthy animals. *Firmicutes* is the major group represented in fecal samples (ranging

Table 2-1 Examples of Biochemical Reactions Performed by the Intestinal Microbiota

Microbial Activity	Products	Representatives
Decarboxylation, deamination of amino acids	Ammonia	*Clostridium* spp., *Peptostreptococcus* spp., *Peptococcus* spp.
Deconjugation/dehydroxylation of bile acids	Secondary bile acids (cholate/deoxycholate)	*Clostridium hiranonis*, *Lactobacillus* spp.
Vitamin synthesis	Vitamins K_2, B_{12}, biotin, folate	*Enterococcus* spp., *Pseudomonas* spp., *Sphingomonas* spp., *Lactobacillus* spp.
Carbohydrate fermentation	Lactate, propionate, acetate, butyrate	*Clostridium* cluster XIVa, *Prevotella* spp., *Faecalibacterium* spp., *Bifidobacterium* spp.
Amino acid fermentation	Hydrogen, methane, amines, phenols, ammonia (NH_3), organic acids, hydrogen sulfite	Sulfate-reducing bacteria (SRB), *Desulfovibrio* spp., *Clostridium* spp., *Peptostreptococcus* spp.
Degradation of oxalate	Formate and CO_2	*Oxalobacter formigenes*
Inulin and starch degradation	Lactate	*Bifidobacterium* spp.
Metabolism of alcohols and acetic acid	Methane and CO_2	Methanobacteria

Table 2-2 Predominant Bacterial Groups in the Canine and Feline Gastrointestinal Tract

CULTURE RESULTS		16S rRNA GENE RESULTS	
Bacterial Group	**Counts (Log CFU/g)**	**Bacterial Group**	**(% of Total Sequences)**
Stomach			
Streptococcus spp.	3.0-5.9	*Helicobacter* spp.	>90
Lactobacillus spp.	1.0-5.4	*Burkholderiales*	<1
Bacteroides	0-4.2	*Clostridiales*	<1
Clostridium perfringens	0-3.2	*Lactobacillales*	<1
Enterobacteriaceae	1.0-3.3	Other	<1
Small Intestine			
Spiral-shaped rods	3.0-6.8	*Clostridiales*	30-50
Bacteroides	0-5.5	*Enterobacteriales*	20-60
Lactobacillus spp.	1.0-5.4	*Lactobacillales*	5-30
Streptococcus spp.	3.0-5.2	*Bacteroidales*	0-5
Escherichia coli	2.3-5.0	*Campylobacterales*	0-2
C. perfringens	1.0-2.5	*Actinomycetales*	0-3
		Fusobacteriales	0-10
		Pasteurellales	2-5
		Spirochaetes	0-12
Large Intestine			
Bacteroides	7.3-10.2	*Clostridiales*	60-78
Bifidobacterium spp.	8.0-10.0	*Lactobacillales*	1-5
Clostridium spp.	7.3-9.5	Erysipelotrichales	0-8
Streptococcus spp.	8.8-9.1	*Bacteroidales*	0.5-5
Lactobacillus spp.	5.5-9.0	*Coriobacteriales*	1-2.5
E. coli	6.4-8.6	*Enterobacteriales*	0.1-2
Prevotella	7.0-8.5	*Fusobacteriales*	0.3-10
Ruminococcus	7.0-8.0	*Aeromonadales*	0.2-0.5
C. perfringens	5.5-8.0	*Bifidobacterium* spp.	N/A
Staphylococcus spp.	5.2-5.3	*Desulfovibrio* spp.	N/A

Results were obtained either by bacterial culture,[1,7] 16S rRNA gene sequencing,[36,48] sequencing of the cpn60 gene,[8] or FISH.[23]

between 30% to 95% of 16S rRNA gene sequences in various studies), followed by *Bacteroides*, *Actinobacteria*, and *Fusobacteria*. *Firmicutes* are a heterogenous bacterial phylum. They are represented mainly by the *Clostridiales* and Erysipelotrichaceae. Within those orders, *Clostridium* spp., *Ruminococcus* spp., *Faecalibacterium* spp., *Dorea* spp., and *Turicibacter* spp. are the major groups. Based on phylogenetic analysis, the *Clostridiales* are comprised of at least 70 different species (Table 2-3), which are organized into phylogenetically distinct *Clostridium* clusters. These clusters differ in abundance in different parts of the intestine. Clusters XIVa and IV encompass many important short-chain fatty acid-producing bacteria (see Table 2-3) and predominate in the ileum and colon of both cats and dogs. Cluster XI and I (*Clostridium perfringens* group) are the second most abundant groups in the small and large intestine of dogs and cats.[6,7]

Presence of a Core Microbiome and Functional Redundancy

There are marked differences in the composition of the microbiota between individuals, and even between monozygotic twins. However, the metabolic end products are similar between individuals. Additionally, although some environmental influences lead to significant changes in bacterial groups, these changes are not immediately associated with any obvious changes in gut function in healthy animals. New metagenomic studies have evaluated the gene content of the intestinal microbiota and suggest that the intestine harbors a "core microbiome," because despite observed differences

Table 2-3 Most Abundant Representative of the Various *Clostridium* Clusters in Canine and Feline Fecal Samples

Cluster I
Clostridium perfringens
Clostridium colicanis
Clostridium disporicum

Cluster IV
Faecalibacterium spp.
Clostridium methylpentosum
Ruminococcus spp.

Cluster XI
Clostridium hiranonis
Clostridium bartlettii
Clostridium lituseburense
Clostridium sordellii
Clostridium glycolicum
Peptostreptococcus spp.

Cluster XIVa
Dorea spp.
Roseburia/Ruminococcus group
Clostridium saccharolyticum
Clostridium celerecrescens
Clostridium symbiosum
Clostridium bolteae
Clostridium oroticum
Clostridium methoxybenzovorans
Clostridium algidixylanolyticum
Clostridium hathewayi
Clostridium amygdalinum
Lachnospiraceae

Cluster XVIII
Clostridium cocleatum
Clostridium ramosum

The order *Clostridiales* is the most abundant and most diverse group in the large intestine of dogs and cats, is comprised of at least 70 known species, and constitutes approximately one-third of total colonic bacteria.

in bacterial phylotypes among individuals, the microbiome of each individual appears to have similar gene content and therefore similar functions.[18] Furthermore, a functional redundancy exists in the GI tract. Several members of the community can perform similar functions, and if one group is displaced because of perturbations (e.g., antibiotic therapy), other members of the community are able to maintain a stable ecosystem. These findings highlight the need to evaluate the intestinal microbiome as an entity, including phylogenetic relationships and metabolic functions (i.e., metagenome, transcriptome, and metabolome).

Other Members of the Intestinal Ecosystem

Besides bacteria, the GI tract harbors fungi, archaea, protozoa, and viruses (including bacteriophages). Recent molecular studies have provided information about the diversity of these microorganisms, but their interactions, their influences on the host, and their role in health and disease remain unclear.

Fungi

Specific fungal organisms (e.g., *Histoplasma capsulatum*) are associated with GI disease, but the role of fungal organisms in the intestinal ecosystem has not been studied extensively. Identification and characterization of fungi is technically challenging. Special staining techniques (e.g., Gomori methenamine silver, Gridley fungus, and periodic acid-Schiff stains) improve the detection sensitivity on histologic sections or fecal smears, but do not allow identification of the organisms.[23] Fungal culture is technically challenging, and serologic tests and immunoassays for the detection of fungal antibodies and antigens are only available for specific pathogens.

The significance of fungi for the GI health of dogs and cats remains unclear. Yeasts and molds have been cultured from the intestine of 25% of healthy Beagles, with mean counts of 10^1 CFU/g jejunal content and 10^5 CFU/g of feces.[20,24,25] A higher prevalence of fungal DNA (76% of dogs) was reported in the proximal small intestine in healthy dogs and dogs with chronic enteropathies using a panfungal PCR assay.[26] A total of 51 different phylotypes were identified in the duodenum of 135 healthy and diseased dogs, with the majority of dogs harboring only one phylotype.[26] Fungi were more frequently adherent to the intestinal mucosa than in the luminal content.[26,27] Recent unpublished data from the author's laboratory obtained using panfungal PCR primers followed by 454-pyrosequencing revealed four fungal phyla in canine and feline fecal samples, with the majority of sequences belonging to Ascomycota (>90%) and *Neocallimastigomycota* (>5%). Saccharomycetaceae were the predominating fungal group in fecal samples of dogs and cats (Table 2-4). All 19 animals evaluated harbored fungal organisms, and multiple species (median, 40; range, 10 to 98) were observed in each sample. Each animal had a unique fungal profile.

Table 2-4 Most Prevalent Fungal Genera Identified in Canine Fecal Samples

Fungal Genus	Mean % of Fungal Population	% of Dogs (n = 19)
Catenulostroma	11.4	94.7
Candida	11.6	94.7
Penicillium	2.8	89.5
Aureobasidium	2.9	84.2
Myrothecium	5.6	78.9
Bipolaris	4.2	78.9
Keissleriella	1.0	73.7
Teratosphaeria	1.1	73.7
Phoma	2.7	73.7
Phomatospora	6.0	73.7
Cochliobolus	7.1	68.4
Cladosporium	5.5	57.9
Pyrenophora	1.6	57.9
Aspergillus	1.3	57.9
Hypocrea	1.2	47.4
Phaeosphaeria	2.4	47.4
Shiraia	1.1	47.4
Saccharomyces	0.8	47.4
Pleiochaeta	0.1	42.1
Engyodontium	0.5	42.1
Nomuraea	0.2	31.6
Alternaria	0.7	31.6
Trematosphaeria	0.3	31.6
Dendryphion	0.3	31.6
Helicoön	0.1	31.6
Sporisorium	0.2	31.6
Fusarium	0.1	31.6
other	27	N/A

Fecal samples from 19 dogs living in various environments were analyzed by fungal tag-encoded FLX amplicon pyrosequencing (fTEFAP). Fungi were present in all 19 samples. Multiple species (median, 40; range, 10 to 98) were observed in each sample, but each dog harbored a unique fungal profile.

Archaea

Archaea are single-celled microorganisms with structure similar to bacteria. They are evolutionarily distinct from bacteria and eukaryotes and form the third domain of life. Archaea are obligate anaerobes living in environments low in oxygen (e.g., water, soil). Archaea are commensal in the intestine of ruminants and have recently been described in the human intestine, with *Methanobacteriales* most commonly reported.[28,29] Recent 16S rRNA gene-based studies in the author's laboratory revealed two distinct archaeal phyla in the intestine of dogs and cats: *Crenarchaeota* and *Euryarchaeota*. Similar to man, Methanobacteria were the most abundant class of archaea.

The role of archaea in GI health and disease remains unclear. Methanogens are associated with periodontal disease in man.[30] They are considered commensal in the GI tract, but they may contribute to pathogenicity through mutualistic interactions with other microbes.[31] One major function of methanogens is the scavenging of various fermentation products produced by other microbes (e.g., CO_2, H_2, alcohols, and acetic acid), resulting in the production of methane and CO_2. The reduction of hydrogen promotes an environment that favors the growth of polysaccharide fermenting bacteria, leading to a higher energy utilization of the diet. For example, higher numbers of methanogenic archaea have been observed in obese people.[29] It has also been hypothesized that reduction in hydrogen concentrations results in lower production of hydrogen sulfite by sulfate reducing bacteria, thus reducing damage to epithelial cells.

Viruses

Knowledge of viral communities in the GI tract of dogs and cats is limited to a few families (including rotavirus, coronavirus, and parvovirus). Recent human studies revealed that the viral community in the GI tract is as diverse as the bacterial, with several hundred different phylotypes.[32] The vast majority of these are bacteriophages. It is likely that a similar viral community is present in the intestine of dogs and cats. It remains technically challenging to characterize the viral community because of their heterogeneity (i.e., DNA

viruses, RNA viruses, single-stranded DNA [ssDNA] viruses). Consequently, a universal approach as used for bacteria and fungi is not feasible. New metagenomic approaches show the most promise for the characterization of intestinal viruses.

Dynamics of the Intestinal Microbiota

Host genetics substantially influence the overall composition of the intestinal microbiota. Environmental factors such as antibiotic administration and dietary changes cause shifts in microbial groups, but these changes are individualized for each animal.[2,33] The qualitative microbial composition is stable over long periods of time, but the proportions of individual bacterial groups may have substantial day-to-day variation. The small intestinal microbiota has greater temporal variation compared with the more diverse large intestine.[25]

Changes in Intestinal Microbiota During Life Stages

The intestine harbors permanently colonizing microorganisms that are acquired at birth and remain largely stable over life. Passing microbes (i.e., swallowed) are present only transiently in the GI tract. Within hours after birth, the sterile intestine becomes colonized by bacteria present in the birth canal, milk, and surrounding environment.[34] Shifts in relative proportions of bacteria occur during postnatal development. In one study, bacterial counts in the small intestine were highest in one-day-old puppies and decreased significantly thereafter. They remained stable at approximately 10^7 CFU/g over the observation period of 42 days.[34] Aerobes predominate in the first weeks of life, but the proportion of anaerobes gradually increases with age, mostly because of an increase in *Bacteroidetes*. The major shifts in microbial populations correlate with dietary changes (i.e., suckling and transition to solid food) and the physiologic development of the host's ability to metabolize dietary substrates (e.g., development of intestinal brush-border enzymes) in the first weeks after birth.[34,35] The microbiota increases in diversity in the first few months of life and remains remarkably stable during adulthood. More pronounced changes are observed in older animals (i.e., dogs older than 11 years), especially in the large intestine.[20] These changes are most likely caused by the changing structure and function of the GI tract with increasing age. Older dogs have more *Clostridium perfringens* and *Streptococcus* spp., and fewer *Bacteroides*, *Bifidobacterium*, and *Lactobacillus* spp.[20] DGGE fingerprinting profiles also cluster according to age, and levels of *Bacteroides* are significantly lower in older dogs.[33]

Mechanisms Regulating the Intestinal Microbiota

Several physiologic mechanisms regulate bacterial colonization in the intestine. Gastric acid, bile, and pancreatic enzymes inactivate most ingested microorganisms. Intestinal motility is an important regulator of bacterial counts in the intestine, as microbes that are not able to adhere to the epithelium will be quickly eliminated. In contrast, higher numbers of bacteria are present in the large intestine as a consequence of stagnant flow of luminal content and the abundance of nutrients. The ileocolic valve together with normal intestinal motility prevents retrograde migration of bacteria from the large into the small intestine.[36] Any changes in these control mechanisms may lead to alterations to community composition or total bacterial count. For example, atrophic gastritis or acid suppressant therapy leads to an increase in duodenal bacterial counts in man.[37] Similarly, dogs with experimentally induced exocrine pancreatic insufficiency (EPI) have increased bacterial number in the proximal small intestine.[38] Anatomical malformations (e.g., strictures, surgically created blind loops) associated with altered motility are a common site of bacterial overgrowth in man.

Role of Microbiota in Immunity, Host Defense, and Energy Regulation

A balanced intestinal ecosystem primes and stimulates the immune system, aids in defense against intestinal pathogens, and provides nutritional benefits to the host. Animals can live well when raised under germ-free conditions. However, morphologic and immunologic differences between germ-free and conventionally raised animals suggest that that the commensal microbiota is a significant contributor to the development and maintenance of gut physiology and immune function.[39] Germ-free animals have an altered mucosal architecture (i.e., thinner lamina propria) and reduced turnover of epithelial cells compared with conventionally raised animals.[39] The underdeveloped immune system is rapidly restored upon introducing bacteria into germ-free mice.[40] Bacteria communicate with the host via Toll-like receptors (TLRs) and dendritic cells. The resident intestinal microbiota is a crucial part of the intestinal barrier that protects the host from invading pathogens. This mechanism is called *colonization resistance*. Proposed defenses include the competition for oxygen, nutrients, and mucosal adhesion sites, and the creation of a physiologically restrictive environment for nonresident bacterial species (e.g., secretion of antimicrobials, alterations in pH, and hydrogen sulfide production).[41] Other crucial parts of the intestinal barrier are the intestinal epithelial cells, protective mucus, and the gut-associated lymphoid tissue (GALT). The increase in diversity of the colonizing microbiota at weaning strengthens the colonization resistance against pathogens. Furthermore, the resident microbiota drives maturation of the intestinal immune system. Younger animals with underdeveloped microbial diversity and immature GALT are dependent on protective colostral antibodies and milk components. Younger animals are typically more susceptible to invading pathogens (e.g., *Campylobacter* spp.). Studies of murine models have suggested that the pattern of microbial colonization in early life may impact on host physiology and colonizing resistance far into adulthood.[42]

Bacteria in the large and small intestine can differ in their contribution to GI health. The large intestinal microbiota is mainly beneficial to the host. Clostridiaceae, *Dorea* spp., Lachnospiraceae, *Ruminococcus* spp., *Faecalibacterium* spp., and *Roseburia* spp. are the predominant bacteria in the colon. The majority of colonic bacteria are anaerobic and their main functions are to produce energy from undigested food and to help in the competitive exclusion of potential pathogens. The slower flow of ingesta and the increased time and availability of nutrients favors microbial diversity in the colon. Bacteria within this ecosystem have developed cooperative strategies to transform the complexity of nutrients to their own and the host's benefit. Colonic bacteria provide digestive enzymes that allow utilization of complex carbohydrates. For example, 8% of the genome of *Bifidobacterium longum* is comprised of genes needed for carbohydrate metabolism.[43] Microbes metabolize sloughed epithelial cells, endogenous mucus, and nondigested substrates that have passed through the small intestine. The latter are predominantly complex carbohydrates, including starch and dietary fiber such as cellulose, pectin, and inulin. The fermentation of these substrates results mainly in the production of short-chain fatty acids (e.g., acetate, propionate, and butyrate) that provide energy for bacterial metabolism and for epithelial cell growth. Up to 7% of the metabolic energy of dogs, and to a lesser extent in cats, is produced by

microbial fermentation.[44] Peptides, amino acids, and intermediate products of microbial metabolism such as ethanol, lactic acid, and succinic acid are further metabolized into short-chain fatty acids. Some members of the *Clostridium* cluster XIVa also utilize lactate for butyrate production, thus preventing lactate accumulation in the human colon. However, colonic microbial metabolism of some substrates, mainly proteins, may also yield toxic intermediates with negative effects on epithelial cells.

Small intestinal bacteria have a more delicate relationship with the host. As a result of increased intestinal motility, they are predominantly adherent to the mucosa. These organisms are an important stimulator of mucosal immunity. Subtle changes in this balance may impact on the health of the host. The predominantly facultative anaerobic bacteria may compete with the host for nutrients and may produce deleterious metabolites. In pigs, up to 6% of dietary energy may be lost to the host as a result of bacterial uptake in the small intestine.[45] Small intestinal microbiota, especially *Lactobacillus* spp. and *Clostridium* spp. (*C. hiranonis* and *C. scindens*) deconjugate bile acids, impairing fat absorption and producing secondary bile acids that may damage the epithelium.[46] Other abnormal functions may be dehydroxylation of fatty acids, destruction of brush-border enzymes, damage of carrier proteins, and competition for nutrients (e.g., cobalamin).

Gastrointestinal Microbiota in Disease

The close contact between microbiota and host has significant impact on GI health. Colonization with transient pathogens, overgrowth of resident opportunistic commensals, or altered communication between the intestinal innate immune system and the commensal microbiota may result in GI disease. Invasion of specific pathogens (i.e., *Salmonella*, enterotoxigenic *C. perfringens*, *Campylobacter jejuni*, and others) may profoundly disturb the structure of the GI mucosa. Enteric pathogens can penetrate into the submucosa and Peyer's patches, or produce exo- or enterotoxins that alter enterocyte function. Enterotoxins often stimulate mucosal fluid secretion, while villus effacement and loss of surface area diminishes mucosal absorptive capacity, resulting in diarrhea. Dysfunction of the mucosal barrier can lead to increased intestinal permeability and clinically significant bacterial translocation.[47]

Several GI diseases are associated with nonspecific alterations in the microbiota. Small intestinal bacterial overgrowth or antibiotic-responsive diarrhea (also known as tylosin-responsive diarrhea) is suspected to be caused by an intestinal dysbiosis.[48] Rapid diet changes or dietary indiscretion, changes in the architecture of the intestine, or changes in intestinal motility (e.g., surgical creation of intestinal loops, short bowel syndrome, and resection of the ileocolic valve) are also associated with alterations in the intestinal ecosystem. EPI is associated with an increase in bacterial counts in the canine small intestine, which often is reversible upon pancreatic enzyme supplementation.[38] Such alterations may lead to various mechanisms that will negatively impact the function of the GI tract. Examples are an altered intestinal barrier with increased intestinal permeability, and direct damage to the intestinal brush-border and enterocytes leading to nutrient and vitamin malabsorption. Overgrowth of specific bacterial groups may lead to increased competition for nutrients and vitamins and increased deconjugation of bile acids and creation of potentially deleterious metabolites.

The commensal intestinal microbiota is also thought to play an integral part in the pathogenesis of inflammatory bowel disease (IBD) in man, dogs, and cats.[1,13,49] In man, the microbiota is implicated because inflammation is present in gut compartments with the highest bacterial counts and the diversion of the fecal stream or antibiotic therapy improves clinical signs. In murine models of IBD, inflammation develops only in the presence of bacteria. The cause–effect relationship between microbial alterations and inflammation is not well determined. It is suspected that intestinal inflammation causes a shift toward Gram-negative bacteria (e.g., *Proteobacteria*) that may perpetuate the disease in genetically susceptible individuals. New hypotheses also suggest that intestinal inflammation may trigger alterations in the immune system, which, in turn, diminish the colonization resistance of the resident microbiota, resulting in an overgrowth of pathogens.[50] One current hypothesis implicates an abnormal interaction between commensal bacteria and the intestinal immune system in genetically predisposed individuals.[51] For example, a subset of people with Crohn's disease may not be able to effectively clear commensal or pathogenic bacteria, resulting in overcompensating antibacterial effector T cells that may, in turn, cause tissue damage.[51] People with Crohn's disease have a decrease in the bacterial phyla *Firmicutes* and *Bacteroidetes*, and an increase in *Proteobacteria* and *Actinobacteria*.[1] In most studies of human IBD, a reduction in the diversity of *Clostridium* clusters XIVa and IV (e.g., *Lachnospiraceae*, *Ruminococcaceae*, *Faecalibacterium prausnitzii* and *Clostridium coccoides* subgroups) was identified, suggesting that these bacterial groups, which are mainly producers of short-chain fatty acids, may play an important role in maintenance of GI health. Alterations in microbial composition have also been recently reported in dogs and cats with IBD.[13,49] Similar to people, dogs and cats with idiopathic IBD had significantly more Proteobacteria and reductions in *Clostridium* clusters XIVa and IV in their duodenum compared with healthy animals.[13,49,52] Boxer dogs with histiocytic ulcerative colitis respond to therapy with fluoroquinolones, and in this disease there is an association between the presence of adherent and invasive *Escherichia coli* (AIEC) and inflammation.[53] These AIEC isolates share similarities to those obtained from ileal tissues of people with Crohn's disease.[53]

Modulating the Intestinal Microbiota

Members of *Clostridium* clusters XIVa and IV (e.g., *Dorea* spp., *Lachnospiraceae*, *Ruminococcus* spp., *Faecalibacterium* spp., and *Roseburia* spp.) are consistently depleted in people with IBD and acute colitis,[51] suggesting that these organisms are important in maintaining intestinal homeostasis. This observation emphasizes the need to distinguish the presence of beneficial clostridial groups from opportunistic commensals such as *C. perfringens* and *Clostridium difficile*. Changes in proposed beneficial bacterial groups such as *Bifidobacterium* spp. have also been observed in GI disease, although to a lesser extent. Modulating the intestinal microbial ecosystem is therefore a rational therapeutic approach in animals with GI disease. Clinical experience shows that dietary changes or antibiotic administration often leads to an improvement in GI signs. However, the exact mechanism remains elusive. Antibiotics can eliminate specific pathogens or can, as is also proposed for diets, lead to more general modulations of the intestinal microbiota. This potentially results in a reduced burden of stimulating antigens or the creation of an environment within the GI tract (e.g., changes in osmolarity and pH) that allows for more effective utilization of ingesta.

Because of vast interindividual differences in the intestinal microbiota, it is difficult to define what constitutes a normal and balanced intestinal ecosystem. Dietary manipulation, the administration of antibiotics or pre- and/or probiotics are commonly used modulating strategies. It is important to note that although such interventions may cause shifts in the composition of the intestinal microbiota, these

changes are largely individualized for each animal. Bacterial groups of a higher phylogenetic level (i.e., order or family) may show a synchronized response to the environmental influence, but these changes are rarely associated with one specific bacterial species or bacterial strain that is consistently altered in every individual. As a consequence of the complexity of the microbiota, the vast majority of bacterial groups within the ecosystem remain typically unaffected by diet or nutraceuticals.[33,54] This complicates the prediction as to which animal may benefit most from a selected strategy. Probiotics can lead to a transient increase in the administered target species, but this has an insignificant impact on the composition of the total microbial ecosystem. Furthermore, probiotics are typically eliminated from the intestine within a few days after ending administration. Therefore administration of high doses over prolonged periods of time is usually required. Prebiotics are typically complex carbohydrates that are added to diets to enhance the growth of endogenous microorganisms; including *Bifidobacterium* spp. Increases in bacterial groups that utilize these nutrients have been demonstrated.[55] Such changes are again typically very minor within the entire ecosystem, as the administered prebiotics fulfill only some of the nutrient requirements for their target bacteria, but other essential nutrients remain at growth-limiting amounts. At this point, the significance of altering a rather minor proportion of the ecosystem remains unclear. It is also now well recognized that the microbiota is generally resilient to change and returns rapidly to its pretreatment state within a few days. Consequently, long-term modulation is needed to maintain a desired ecosystem. Antibiotic usage has a more pronounced effect and may disrupt the microbial ecosystem for prolonged periods of time (weeks to months). In one study evaluating the fecal microbiota of healthy people, approximately 30% of all bacterial taxa were affected, some of them for up to six months.[11] Similarly, administration of tylosin for 14 days led to significant modifications in the jejunal microbiota of dogs, with some bacterial groups depressed for more than 14 days.[2] However, highly individualized responses for some bacterial groups were observed in each animal.

Based on these findings, it remains challenging to provide a universal algorithm for modulation of the microbiota. Every animal may require individualized management consisting of combinations of dietary modification, antibiotics, and pro- or prebiotics. Therefore, therapeutic modulation is currently based on empirical approaches with improvement of clinical signs as the most useful outcome measure. New molecular tools such as metagenomics and transcriptomics will be useful for elucidating how such modulatory strategies affect the gene content within the intestinal microbiome. This may help to customize treatment strategies for individual animals and conditions.

References

1. Frank DN, Amand ALS, Feldman RA, et al: Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci U S A* 104:13780, 2007.
2. Suchodolski JS, Dowd SE, Westermarck E, et al: The effect of the macrolide antibiotic tylosin on microbial diversity in the canine small intestine as demonstrated by massive parallel 16S rDNA sequencing. *BMC Microbiol* 10:210, 2009.
3. Ferrari BC, Winsley T, Gillings M, et al: Cultivating previously uncultured soil bacteria using a soil substrate membrane system. *Nat Protoc* 3:1261, 2008.
4. Greetham HL, Giffard C, Hutson RA, et al: Bacteriology of the Labrador dog gut: a cultural and genotypic approach. *J Appl Microbiol* 93:640, 2002.
5. Dethlefsen L, Eckburg PB, Bik EM, et al: Assembly of the human intestinal microbiota. *Trends Ecol Evol* 21:517, 2006.
6. Ritchie LE, Steiner JM, Suchodoiski JS: Assessment of microbial diversity along the feline intestinal tract using 16S rRNA gene analysis. *FEMS Microbiol Ecol* 66:590, 2008.
7. Suchodolski J, Camancho J, Steiner J: Analysis of bacterial diversity in the canine duodenum, jejunum, ileum, and colon by comparative 16S rRNA gene analysis. *FEMS Microbiol Ecol* 66:567, 2008.
8. Zoetendal EG, Cheng B, Koike S, et al: Molecular microbial ecology of the gastrointestinal tract: from phylogeny to function. *Curr Issues Intest Microbiol* 5:31, 2004.
9. Forney LJ, Zhou X, Brown CJ: Molecular microbial ecology: land of the one-eyed king. *Curr Opin Microbiol* 7:210, 2004.
10. Desai AR, Musil KM, Carr AP, et al: Characterization and quantification of feline fecal microbiota using cpn60 sequence-based methods and investigation of animal-to-animal variation in microbial population structure. *Vet Microbiol* 28:120, 2008.
11. Dethlefsen L, Huse S, Sogin ML, et al: The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol* 6:e280, 2008.
12. Lubbs DC, Vester BM, Fastinger ND, et al: Dietary protein concentration affects intestinal microbiota of adult cats: a study using DGGE and qPCR to evaluate differences in microbial populations in the feline gastrointestinal tract. *J Anim Physiol Anim Nutr (Berl)* 93:113, 2009.
13. Janeczko S, Atwater D, Bogel E, et al: The relationship of mucosal bacteria to duodenal histopathology, cytokine mRNA, and clinical disease activity in cats with inflammatory bowel disease. *Vet Microbiol* 128:178, 2008.
14. Lipski A, Friedrich U, Altendorf K: Application of rRNA-targeted oligonucleotide probes in biotechnology. *Appl Microbiol Biotechnol* 56:40, 2001.
15. Sghir A, Gramet G, Suau A, et al: Quantification of bacterial groups within human fecal flora by oligonucleotide probe hybridization. *Appl Environ Microbiol* 66:2263, 2000.
16. Rastogi R, Wu M, DasGupta I, et al: Visualization of ribosomal RNA operon copy number distribution. *BMC Microbiol* 9:208, 2009.
17. Zoetendal EG, Collier CT, Koike S, et al: Molecular ecological analysis of the gastrointestinal microbiota: a review. *J Nutr* 134:465, 2004.
18. Turnbaugh PJ, Hamady M, Yatsunenko T, et al: A core gut microbiome in obese and lean twins. *Nature* 457:480, 2009.
19. Suchodolski JS, Ruaux CG, Steiner JM, et al: Assessment of the qualitative variation in bacterial microflora among compartments of the intestinal tract of dogs by use of a molecular fingerprinting technique. *Am J Vet Res* 66:1556, 2005.
20. Benno Y, Nakao H, Uchida K, et al: Impact of the advances in age on the gastrointestinal microflora of beagle dogs. *J Vet Med Sci* 54:703, 1992.
21. Elliott DR, Wilson M, Buckley CMF, et al: Cultivable oral microbiota of domestic dogs. *J Clin Microbiol* 43:5470, 2005.
22. Johnston KL, Lamport A, Batt RM: An unexpected bacterial flora in the proximal small intestine of normal cats. *Vet Rec* 132:362, 1993.
23. Dupont B: An epidemiological review of systemic fungal infections. *J Mycol Med* 12:163, 2002.
24. Davis CP, Cleven D, Balish E, et al: Bacterial association in the gastrointestinal tract of beagle dogs. *Appl Environ Microbiol* 34:194, 1977.
25. Mentula S, Harmoinen J, Heikkila M, et al: Comparison between cultured small-intestinal and fecal microbiotas in Beagle dogs. *Appl Environ Microbiol* 71:4169, 2005.
26. Suchodolski J, Morris E, Allenspach K, et al: Prevalence and identification of fungal DNA in the small intestine of healthy dogs and dogs with chronic enteropathies. *Vet Microbiol* 132:379, 2008.

27. Scupham AJ, Presley LL, Wei B, et al: Abundant and diverse fungal microbiota in the murine intestine. *Appl Environ Microbiol* 72:793, 2006.
28. Eckburg PB, Bik EM, Bernstein CN, et al: Diversity of the human intestinal microbial flora. *Science* 308:1635, 2005.
29. Zhang HS, DiBaise JK, Zuccolo A, et al: Human gut microbiota in obesity and after gastric bypass. *Proc Natl Acad Sci U S A* 106:2365, 2009.
30. Li CL, Liu DL, Jiang YT, et al: Prevalence and molecular diversity of Archaea in subgingival pockets of periodontitis patients. *Oral Microbiol Immunol* 24:343, 2009.
31. Conway dM, Macario AJ: Methanogenic archaea in health and disease: A novel paradigm of microbial pathogenesis. *Int J Med Microbiol* 299:99, 2008.
32. Breitbart M, Hewson I, Felts B, et al: Metagenomic analyses of an uncultured viral community from human feces. *J Bacteriol* 185:6220, 2003.
33. Simpson JM, Martineau B, Jones WE, et al: Characterization of fecal bacterial populations in canines: effects of age, breed and dietary fiber. *Microb Ecol* 44:186, 2002.
34. Buddington RK: Postnatal changes in bacterial populations in the gastrointestinal tract of dogs. *Am J Vet Res* 64:646, 2003.
35. Buddington RK, Elnif J, Malo C, et al: Activities of gastric, pancreatic, and intestinal brush-border membrane enzymes during postnatal development of dogs. *Am J Vet Res* 64:627, 2003.
36. Griffen WO Jr, Richardson JD, Medley ES: Prevention of small bowel contamination by ileocecal valve. *South Med J* 64:1056, 1971.
37. Camilo E, Zimmerman J, Mason JB, et al: Folate synthesized by bacteria in the human upper small intestine is assimilated by the host. *Gastroenterology* 110:991, 1996.
38. Simpson KW, Batt RM, Jones D, et al: Effects of exocrine pancreatic insufficiency and replacement therapy on the bacterial flora of the duodenum in dogs. *Am J Vet Res* 51:203, 1990.
39. Falk PG, Hooper LV, Midtvedt T, et al: Creating and maintaining the gastrointestinal ecosystem: what we know and need to know from gnotobiology. *Microbiol Mol Biol Rev* 62:1157, 1998.
40. Imaoka A, Matsumoto S, Setoyama H, et al: Proliferative recruitment of intestinal intraepithelial lymphocytes after microbial colonization of germ-free mice. *Eur J Immunol* 26:945, 1996.
41. Kanauchi O, Matsumoto Y, Matsumura M, et al: The beneficial effects of microflora, especially obligate anaerobes, and their products on the colonic environment in inflammatory bowel disease. *Curr Pharm Des* 11:1047, 2005.
42. Schaedler RW, Dubos RJ: Fecal flora of various strains of mice—its bearing on their susceptibility to endotoxin. *J Exp Med* 37:1149, 1962.
43. Louis P, Scott KP, Duncan SH, et al: Understanding the effects of diet on bacterial metabolism in the large intestine. *J Appl Microbiol* 102:1197, 2007.
44. Stevens CE, Hume ID: Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. *Physiol Rev* 78:393, 1998.
45. Vervaeke IJ, Decuypere JA, Dierick NA, et al: Quantitative in vitro evaluation of the energy-metabolism influenced by virginiamycin and spiramycin used as growth promoters in pig nutrition. *J Anim Sci* 49:846, 1979.
46. Kitahara M, Takamine F, Imamura T, et al: *Clostridium hiranonis* sp. nov., a human intestinal bacterium with bile acid 7alpha-dehydroxylating activity. *Int J Syst Evol Microbiol* 51:39, 2001.
47. Guarner F: Enteric flora in health and disease. *Digestion* 73:5, 2006.
48. Westermarck E, Skrzypczak T, Harmoinen J, et al: Tylosin-responsive chronic diarrhea in dogs. *J Vet Intern Med* 19:177, 2005.
49. Suchodolski JS, Xenoulis PG, Paddock CG, et al: Molecular analysis of the bacterial microbiota in duodenal biopsies from dogs with idiopathic inflammatory bowel disease. *Vet Microbiol* 2010;142:394.
50. Stecher B, Hardt WD: The role of microbiota in infectious disease. *Trends Microbiol* 16:107, 2008.
51. Packey CD, Sartor RB: Commensal bacteria, traditional and opportunistic pathogens, dysbiosis and bacterial killing in inflammatory bowel diseases. *Curr Opin Infect Dis* 22:292, 2009.
52. Xenoulis P, Palculict B, Allenspach K, et al: Molecular-phylogenetic characterization of microbial communities imbalances in the small intestine of dogs with inflammatory bowel disease. *FEMS Microbiol Ecol* 66:579, 2008.
53. Simpson KW, Dogan B, Rishniw M, et al: Adherent and invasive *Escherichia coli* is associated with granulomatous colitis in boxer dogs. *Infect Immun* 74:4778, 2006.
54. Lappin MR, Veir JK, Satyaraj E, et al: Pilot study to evaluate the effect of oral supplementation of *Enterococcus faecium* SF68 on cats with latent feline herpesvirus 1. *J Feline Med Surg* 11:650, 2009.
55. Gibson GR, Roberfroid MB: Dietary modulation of the human colonic microbiota—introducing the concept of prebiotics. *J Nutr* 125:1401, 1995.