COMPANION ANIMALS SYMPOSIUM: Role of microbes in canine and feline health

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ABSTRACT: Whether in an ocean reef, a landfill, or a gastrointestinal tract (GIT), invisible communities of highly active and adaptable microbes prosper. Over time, mammals have developed a symbiosis with microbes that are important inhabitants not only in the GIT, but also in the mouth, skin, and urogenital tract. In the GIT, the number of commensal microbes exceeds the total number of host cells by at least 10 times. The GIT microbes play a critical role in nutritional, developmental, defensive, and physiologic processes in the host. Recent evidence also suggests a role of GIT microbes in metabolic phenotype and disease risk (e.g., obesity, metabolic syndrome) of the host. Proper balance is a key to maintaining GIT health. Balanced microbial colonization is also important for other body regions such as the oral cavity, the region with the greatest prevalence of disease in dogs and cats. A significant obstruction to studying microbial populations has been the lack of tools to identify and quantify microbial communities accurately and efficiently. Most of the current knowledge of microbial populations has been established by traditional cultivation methods that are not only laborious, time-consuming, and often inaccurate, but also greatly limited in scope. However, recent advances in molecular-based techniques have resulted in a dramatic improvement in studying microbial communities. These DNA-based high-throughput technologies have enabled us to more clearly characterize the identity and metabolic activity of microbes living in the host and their association with health and diseases. Despite this recent progress, however, published data pertaining to microbial communities of dogs and cats are still lacking in comparison with data in humans and other animals. More research is required to provide a more detailed description of the canine and feline microbiome and its role in health and disease.

Key words: cat, cultivation method, dog, health, microbe, molecular-based technique

INTRODUCTION

Microbes are the smallest of all life forms and are spread over the world as a highly dense and diverse population. Likewise, the human and animal body, including the gastrointestinal tract (GIT), mouth, skin, and urogenital tract harbors complex microbial communities. The primary region of microbial colonization in humans and animals is the GIT (Savage, 1977). It has been estimated that approximately $10^{13}$ cfu of bacteria composed of 500 to 1,000 different species reside in the GIT (Manson et al., 2008). It is now apparent that the symbiotic residence of the GIT microbes plays a critical role in a wide array of nutritional, developmental, defensive, and physiologic processes in humans and animals (Mackie et al., 1999). Similar host-microbe interactions are also expected in other body regions of the host.

The primary tasks in the study of microbial communities are to explore 1) what microbes live in a desirable microbial community, 2) what the roles of microbes are in a desirable microbial community, and 3) the means to manipulate the microbial population toward developing a desirable microbial community. Most of the current knowledge pertaining to microbial communities has been obtained from cultivation methods, despite their inaccuracies and limitation in scope. Recently, various molecular-based techniques have been developed and applied to microbial studies, which has greatly expanded our understanding of the complex microbial ecosystems in humans and animals (Zoetendal et al., 2004a,b).
Many more papers have been published about humans, rodents, and livestock, however, than papers pertaining to canine and feline microbes characterized by cultivation methods or molecular-based techniques.

The objectives of this review were to describe the known physiological roles of microbes in health and disease, the traditional and emerging methodologies used to characterize microbial composition and dynamics, and how these techniques have improved our knowledge of the microbial populations in dogs and cats.

**IMPORTANCE OF MICROBES IN THE HOST**

The microbial community, or microbiota, in the host has been implicated in a wide variety of biological functions. In humans and animals, the GIT is arguably the most important interface between host and microbes (Savage, 1977). Therefore, most previous investigations on the physiological roles of the microbiota have focused on the GIT. Evidence from germ-free animals or animals reared in a sterile environment has revealed the critical role of GIT microbes in host physiology. For instance, germ-free animals have been described to have increased requirements for energy and vitamins B and K, impaired intestinal structure and morphology, delayed gastric motility, and decreased immune defense as compared with conventional animals, indicating that microbes are an essential component in normal GIT development and function (Tannock, 1997). Beyond the function of microbes in the GIT, it is now appreciated that differences in microbial composition systemically affect host metabolic phenotypes such as obesity (Turnbaugh et al., 2006), diabetes, and nonalcoholic hepatosteatosis (Dumas et al., 2006). Although the mechanisms underlying how microbes are involved in these host physiological changes are unclear, microbial metabolic activity and its metabolites (e.g., short-chain fatty acids) are potential mediators (Tannock, 1997; Dumas et al., 2006; Turnbaugh et al., 2006).

In all biological ecosystems, there are continuous microbe-microbe interactions (Dolfing and Gottschal, 1997). Whereas many microbes cooperate with one another for survival, others are in direct competition for available nutrients and space or producing antimicrobial agents (Dolfing and Gottschal, 1997), a process referred to as competitive exclusion (Hardin, 1960). These interactions are an important feature of the host defensive mechanism against pathogen colonization. It has been noted that germ-free animals and young individuals with an immature community of resident microbes are highly susceptible to the invasion of pathogenic microbes, which implies that resident microbes play a key role in maintaining microbial homeostasis by preventing the colonization by pathogenic or nonresidential microbes in the GIT (Rolfe, 1997). Although little is known about the physiological role of resident microbes in other regions of the host, the maintenance of a balanced microbial population appears to be of clinical importance as observed in the GIT.

**CANINE AND FELINE DISEASES INFLUENCED BY MICROBES**

In humans and animals, body regions have a distinct quantity and composition of microbes. In general, microbial communities in the body can be divided into commensal (i.e., normal) resident microbes and pathogenic microbes. The discrimination between commensal and pathogenic microbes is often difficult. Although many microbes are correlated with illness, one must determine whether a microbe is a contributor or simply one that benefits from the conditions of a diseased environment. Several potential pathogenic bacteria related to canine and feline infections are presented in Table 1. It should be noted, however, that many of these microbes are also present in healthy dogs and cats. Thus, microbial balance or activity or both appear to be more important than the mere presence of any 1 pathogenic microbe. This section will review the current knowledge pertaining to oral, GIT, urogenital, and skin microbiota and health, most of which has been described using traditional assays.

**Oral Cavity**

The healthy canine and feline oral cavity contains approximately 10^7 cfu/mL of fluid, containing a diverse population of aerobic and anaerobic bacteria (Greene, 1998). Continuous licking behavior, high moisture content, and exposure to the environment contribute to the large number of oral microbes. Oral diseases are often the most commonly reported disorders in adult dogs and cats (Lund et al., 1999). More than 80% of dogs >2 yr old are reported to suffer from some type of periodontal disease (Harvey, 1998). As compared with normal oral microbial populations in dogs, the number of aerobic and facultative anaerobic bacteria is decreased and the number of anaerobic bacteria is increased with periodontal diseases (Greene, 1998). In both canine and feline cases of gingivitis, the gram-negative anaerobic bacteria and the gram-positive aerobes are dominant (Harvey et al., 1995). Specifically, the outgrowth of Spirochetes (e.g., *Treponema*, *Porphyromonas*, *Streptococcus*, and *Fusobacterium* have been often observed in periodontal diseases of dogs and cats (Greene, 1998; Harvey, 1998, 2005).

**GIT**

The stomach of dogs and cats contains a smaller number of microbes (10^3 to 10^5 cfu/g of content; Benno et al., 1992) than the oral cavity because of the acidic conditions of the stomach, which is unfavorable for most microbes. The number of aerobic bacteria is similar to that of anaerobic bacteria (Benno et al., 1992), and the
gram-positive bacteria predominate (Greene, 1998). As observed in humans, *Helicobacter* spp. (e.g., *H. felis, H. bizzozeronii, H. salomonis*) are considered potential pathogens contributing to gastritis and peptic ulcer in dogs and cats (Fox, 1998; Jergens et al., 2009), although its pathological role is not clear in these species (Neiger and Simpson, 2000). In a series of gastric biopsy studies using 112 dogs and 127 cats, 82% of dogs and 76% of cats had *Helicobacter*-like organisms; however, the relationship between *Helicobacter* colonization and indices of gastric inflammation was more pronounced in cats than in dogs (Hermanns et al., 1995).

In the small intestine of dogs and cats, total bacterial number gradually increases from duodenum and jejunum (10^6 to 10^8 cfu per g of content) toward the end of the ileum (10^7 cfu/g of content; Benno et al., 1992; Johnston, 1999). *Eubacterium, Bacteroides, Clostridium, Fusobacterium, Bifidobacterium, and Lactobacillus* are the most common anaerobic bacteria, whereas *Streptococcus, Staphylococcus, Pasteurella, Escherichia*, and *Enterobacter* are the most common aerobic and facultative anaerobic bacteria in dogs and cats (Davis et al., 1977; Benno et al., 1992; Johnston, 1999). The number of aerobic and facultative anaerobic bacteria is similar to that of anaerobic bacteria in the jejunum of dogs (Mentula et al., 2005). In the small intestine of dogs and cats, *Clostridium perfringens, Clostridium difficile, Klebsiella pneumoniae, Campylobacter jejuni, Salmonella typhimurium, enteric Helicobacter* spp., *Yersinia enterocolitica*, and enteropathogenic *Escherichia coli* have been identified as potential pathogenic bacterial species (Greene, 1998; Marks and Kather, 2003). When these pathogenic bacteria attach to the intestinal epithelial surface, they invade mucosal layers or produce enterotoxins or both, which often result in inflammatory bowel diseases and diarrhea (Greene, 1998).

Moreover, although the exact cause of the disease is unknown, small intestinal bacterial overgrowth (SIBO) is a pathogenic syndrome characterized by an abnormal proliferation of bacteria in the duodenum and jejunum of humans and animals (Johnston, 1999). An increased incidence of SIBO has been documented in both dogs, especially the German Shepherd Dog breed, and cats, with the common complication of SIBO being small-bowel diarrhea and steatorrhea (Johnston, 1999). The traditional diagnosis of canine SIBO is based on quantitative bacteria culture and is defined as having >10^5 cfu per g of content of total bacteria in the upper small intestine (Greene, 1998). However, Johnston (1999) recommended that the minimal values for diagnosing SIBO should be increased to at least 10^9 cfu in dogs and 10^8 cfu in cats because healthy dogs and cats often have duodenal and jejunal bacterial number >10^9 cfu (Johnston et al., 1993; Johnston, 1999). Increased populations of *E. coli, Enterococcus, and Clostridium* have been described in dogs with SIBO (Greene, 1998).

The large intestine (cecum and colon) in dogs and cats harbors the largest number of microbes, with populations in the range of 10^9 to 10^10 cfu per g of content (Greene, 1998). More than 30 yr ago, 27 genera consisting of 84 species of bacteria and 5 genera of fungi were cultured in cecum and colon samples of dogs (Davis et al., 1977). The gram-negative aerobic bacteria and spore- or non-spore-forming anaerobic bacteria appear to predominate (Greene, 1998). Although many of the microbes living in the small intestine are also detected in the large intestine, the prevalence of the main microbial groups is likely to differ between these 2 GIT regions (Mentula et al., 2005). For example, anaerobic bacteria including *Eubacterium, Bacteroides, Clostridium, Peptococcus, Bifidobacterium, and Lactobacillus* constitute >90% of the total microbes (Davis et al., 1977; Benno et al., 1992; Greene, 1998), and *Streptococcus* is the primary facultative anaerobic bacteria in the large intestine and feces of dogs (Davis et al., 1977). Many pathogenic bacteria detected in the small intesti-

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**Table 1. Potential pathogenic bacteria present in dogs and cats**

<table>
<thead>
<tr>
<th>Body site</th>
<th>Pathogenic bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouth</td>
<td>Fusobacterium necrophorum and Fusobacterium pseudonecrophorum; Porphyromonas gulae and Porphyromonas gingivalis; Streptococci pyogenes, Streptococci mitis, and Streptococci faecalis; Treponema denticola and Treponema socranskii</td>
</tr>
<tr>
<td>GIT</td>
<td>Anaerobiaspirillum spp.; Bacillus cereus; Campylobacter jejuni and Campylobacter coli; Clostridium perfringens and Clostridium difficile; Enteropathogenic Escherichia coli; Helicobacter felis, Helicobacter bizzozeronii, and Helicobacter salomonis; Klebsiella pneumonia; Salmonella spp.; Yersinia spp.</td>
</tr>
<tr>
<td>Urogenital tracts</td>
<td>Enterobacter spp.; Escherichia coli; Klebsiella spp.; Pasteurella spp.; Proteus spp.; Pseudomonas spp.; Staphylococcus spp.; Staphylococcus aureus, Staphylococcus epidermidis, and Staphylococcus intermedius; Yersinia pestis</td>
</tr>
<tr>
<td>Skin</td>
<td>Actinomyces spp.; Corynebacterium spp.; Escherichia coli; Mycobacterium spp.; Proteus mirabilis; Pseudomonas aeruginosa; Staphylococcus aureus, Staphylococcus epidermidis, and Staphylococcus intermedius; Yersinia pestis</td>
</tr>
</tbody>
</table>

1According to Greene (1998).

2Gastrointestinal tract, including the stomach, small intestine, and large intestine.
tine are also cultured in the large intestine (e.g., *C. perfringens*, *E. coli*, *Y. enterocolitica*) and are expected to exert common pathological actions in the large intestine (Marks and Kather, 2003). Acute and chronic colitis have been documented in dogs and cats, with the colonic inflammation possibly being due to bacterial infection (Greene, 1998). However, there has been no clear evidence as to which specific bacteria are directly involved in the development of colitis.

**Urinary and Genital Tract**

Dogs and cats have an increased incidence of bacterial infections in their urinary or genital tracts or both. More than 14% of all dogs experience a urinary tract infection during their lifetime (Barsanti, 1998), with the clinical incidence of bacterial infection being greater in dogs than in cats (Wooley and Blue, 1976). Male cats are more prone to bacterial infection than female cats (Lees, 1996; Wooley and Blue, 1976); whereas dogs have similar incidence between sexes (Wooley and Blue, 1976). Urinary tract infections are frequently accompanied by genital tract infection and vice versa (Barsanti, 1998). A variety of bacteria including *Streptococcus*, *Staphylococcus*, *E. coli*, *Pseudomonas*, and *Pasteurella* have been found in the urinary and genital tracts of healthy dogs and cats (Barsanti, 1998). However, the abnormal overgrowth of these bacteria or a single bacterium appears to be a primary cause of urinary and genital tract infections. The most common bacteria that proliferate in dogs and cats with urinary tract infections are *E. coli*, *Streptococcus* spp., and *Staphylococcus* spp., whereas *Enterobacter*, *Proteus*, *Klebsiella*, *Pasteurella*, and *Pseudomonas* are detected in some cases (Wooley and Blue, 1976; Lees, 1996; Barsanti, 1998). Bacterial numbers >10^5 cfu/mL of urine have traditionally been used to diagnose urinary tract infections in dogs and cats (Wooley and Blue, 1976; Barsanti, 1998).

**Skin**

A relatively small number of bacteria (less than 350 organisms per cm²) inhabit the skin and hair follicles of dogs and cats (Ihrke, 1998). Resident bacteria (e.g., *Staphylococcus*, *Micrococcus*, α-hemolytic *Streptococcus*, *Clostridium*, *Propionibacterium*, and *Acinetobacter*) and transient bacteria (e.g., *E. coli*, *Proteus*, *Pseudomonas*, and *Bacillus*) have been cultured in the skin of dogs and cats (May, 2006). For bacterial-linked skin diseases of dogs and cats, *Staphylococcus* spp., most commonly *S. intermedius* and less commonly *S. aureus*, have been identified as a primary pathogen (May, 2006), with these bacteria leading to secondary infections of other bacteria such as *Proteus*, *Pseudomonas*, and *E. coli* (Ihrke, 1998). Other pathogenic bacteria such as *Dermatophilus congolensis*, *Mycobacterium* spp., *Actinomyces* spp., and *Yersinia pestis* have been associated with feline facial skin disease, but the cases are rare (Friberg, 2006). Otitis externa is one of the most common skin diseases of dogs and cats and is likely a result of increased colonization by *Staphylococcus intermedius*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *E. coli*, and *Corynebacterium* spp. in the external ear canal of dogs and cats (Rosser, 2004).

**TECHNIQUES FOR CHARACTERIZING MICROBIAL COMMUNITIES**

Cultivation methods have been traditionally used for identifying and enumerating microbes. In fact, most of the current knowledge regarding canine and feline microbial populations, as described in the previous section, has been based on cultivation methods. Nonetheless, cultivation methods are very laborious, time-consuming, and require immediate processing, special handling, or both to maintain the viability of bacteria. More importantly, this method is limited greatly in the scope of microbial identification and differentiation because of the lack of information regarding the growth requirements of selected microbes (Davis et al., 1977; Zoetendal et al., 2004b). As a consequence, only a minor fraction of microbes can be isolated and differentiated in culture. Therefore, the characterization of a microbial community by cultivation methods alone significantly underestimates its diversity and dynamics. It has been estimated that cultivation methods identify <1% of total microbes in water and soils (Amann et al., 1995), and only 20% of total microbes in the GIT of humans (Eckburg et al., 2005). Thus, alternative methods have been needed to obtain a greater understanding of host-microbe interrelationships.

To overcome the limitations of cultivation methods, molecular-based techniques have been increasingly applied to many microbial studies. The primary target for these techniques has been small subunit ribosomal RNA (16S rRNA) because it is ubiquitously present in all microbes and contains both conserved and variable sequence regions across species of bacteria and archaea (Claridge, 2004). Currently, the 16S rRNA sequence is appreciated as a molecular barcode for microbial identity. Therefore, most molecular-based techniques for microbial identification and determination of phylogenetic relationships are largely dependent on 16S rRNA analysis (Claridge, 2004). The 16S rRNA sequence database has grown exponentially since 1990 (Tringe and Hugenholtz, 2008). As of this writing, more than 1,379,000 16S rRNA sequences had been deposited in the 16S rRNA database (http://rdp.cme.msu.edu). The data for the 16S rRNA sequence obtained from the GIT content of human and animals have revealed that most of the GIT microbes have been uncultivated previously, especially for the low G+C gram-positive phyla, due to the limitation in cultivation methods (Zoetendal et al., 2004b). A brief overview of some of the more common molecular-based methods is provided below. A more de-
tailed description of such methods can be found in previous publications (Zoetendal et al., 2004a,b; Richards et al., 2005; Andoh et al., 2009).

Denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) are gel-based methods of fingerprinting, processes that separate 16S rRNA amplicons based on sequence differences (i.e., G + C content) in a polyacrylamide gel. These methods have been used for generating qualitative differences in microbial ecology, especially in response to various host and environmental factors. Suchodolski et al. (2005) analyzed PCR-DGGE profiles of duodenum, jejunum, ileum, colon, and rectum samples of dogs and reported that microbial composition of each GIT region differed among dogs and marked variation also existed in the different GIT regions within individual dogs. As observed in other animals (Richards et al., 2005), Suchodolski et al. (2005) also reported that microbial composition was much more diverse in the lower regions compared with the upper regions of the GIT. Likewise, Simpson et al. (2002) reported that fecal microbial diversity analyzed by DGGE was affected by the age and breed of dogs. Our laboratory also monitored DGGE band patterns from fecal samples of cats fed 2 different concentrations of dietary protein (moderate vs. high) and found that microbial populations were markedly changed in both groups of cats as compared with the baseline, with the differences being more pronounced in cats fed high dietary protein (Lubbs et al., 2009). Because DGGE analysis only provides an estimate of diversity, it is often followed by the analysis of individual DGGE bands for sequence identification or by quantitative PCR (qPCR) or both for quantification.

Quantitative PCR has been widely used for microbial quantification in dogs and cats over the past decade. This approach is very sensitive and is based on the measure of 16S rRNA gene copies, having a sequence unique to the microbe of interest (Richards et al., 2005). Using qPCR techniques, our laboratory has reported the effects of numerous dietary treatments on Bifidobacterium, Lactobacillus, E. coli, and C. perfringens populations in canine and feline fecal samples (Middelbos et al., 2007; Barry et al., 2009, 2010; Lubbs et al., 2009; Vester et al., 2009). Although this method is sensitive and allows for quantification, it is not easily used to evaluate a large number of microbial groups.

As compared with DGGE, the 16S rRNA microarray technique (i.e., Phylochip) may be used as a convenient and high-throughput method for studying microbial communities (Zhou, 2003). This technique is based on the complementary hybridization of microbial 16S rRNA sequences to specific probes spotted on the microarray. Therefore, a single microarray containing a large number of immobilized oligonucleotides targeted to specific microbial sequences permits a large-scale quantitative and phylogenetic analysis of a microbial community (Guschin et al., 1997; Zhou, 2003; Palmer et al., 2006). One weakness is that this technique is only useful for those bacteria with known 16S rRNA sequence. This method has been applied to a variety of microbial ecosystems such as natural environments (Wilson et al., 2002; Zhou, 2003) and the GIT of humans (Palmer et al., 2006; Cox et al., 2010). To our knowledge, however, 16S rRNA microarray analysis for microbial populations in dogs and cats has not been performed previously.

New DNA sequencing techniques have dramatically increased the speed by which microbial communities can be characterized. Traditional Sanger sequencing methods were used to generate the initial 16S rRNA sequence data. This method has greatly improved over time and now generates long sequences (800 to 1,000 bp), allowing for microbial identity with a relatively high confidence (Petrosino et al., 2009). Using Sanger sequencing and comparative 16S rRNA gene analysis, Ritchie et al. (2008) found that Firmicutes (68%) were the most predominant phylum, followed by Proteobacteria (14%), Bacteroidetes (10%), Fusobacteria (5%), and Actinobacteria (4%) in the feline GIT. A similar population distribution was observed using such techniques to describe the healthy canine GIT (Suchodolski et al., 2008). In a similar study comparing healthy dogs vs. dogs with inflammatory bowel disease, it was reported that diseased dogs had a significantly less intestinal microbial diversity, with a decreased percentage of Bacteroidetes and Spirochaetes phyla and an increased percentage of Enterobacteriaceae and Clostridiaceae families (Xenoulis et al., 2008).

Despite the strengths of the Sanger sequencing methods, it is limited in throughput and cost compared with more recently developed sequencing techniques. Approximately 500 bp of 16S rRNA sequence containing several variable regions is capable of providing an adequate proxy for the full-length sequence used for microbial identification and classification (Claridge, 2004). Therefore, recent DNA sequencing techniques such as 454 pyrosequencing that generate sequence lengths of approximately 450 bp and are much faster and cheaper than the Sanger sequencing method, have quickly been applied to microbiological studies (Petrosino et al., 2009). Although it is not as consistent and generates shorter sequences than the Sanger method, the read length by pyrosequencing continues to increase and is expected to be of similar read length in the near future. Therefore, this method enables the efficient study of microbial DNA sequence data on a large scale and in terms of phylogeny (e.g., who is there?) and metagenomic (what are they doing?) analyses. The human microbiome project (HMP) is an excellent example of how to apply high throughput DNA pyrosequencing to human and animal health. In May 2007, the National Institutes of Health (NIH) roadmap initiated the HMP to characterize the entire community of microbiota inhabiting the human GIT, mouth, vagina, nasal cavity, skin, and many other body regions to determine the unique and complex microbial structures of each body region and relate it to human health and disease (Peterson et al., 2009). The pyrosequencing method is
a core technique being used to determine the microbial diversity and relatedness, and even for taxonomic assignment in the HMP (Hamady and Knight, 2009). This method is also essential for metagenomic analyses of complex microbial communities in the HMP. Although the focus of the HMP is human health and disease, the knowledge gained from the microbial sequencing projects and projects studying host-microbe relationships under this initiative will also benefit those studying companion and livestock animals. Whereas the use of high throughput pyrosequencing techniques is rapidly growing in human microbial profiling, this method has rarely been used for such objectives in dogs and cats.

Our laboratory, however, was the first to investigate both the phylogenetic and metagenomic characteristics of fecal microbiota of dogs (Middelbos et al., 2010; Swanson et al., 2010) and cats (Barry, 2010) using high-throughput pyrosequencing techniques. Middelbos et al. (2010) detected the presence of 7 bacterial phyla including Actinobacteria, Firmicutes, Bacteroidetes, Fusobacteria, Proteobacteria, Spirochaetes, and Tenericutes in healthy adult dogs. Compared with the hindgut of humans whereby Firmicutes and Bacteroidetes are the predominant phyla (more than 90%, Turnbaugh et al., 2007), the hindgut of dogs showed a codominant presence of Fusobacteria equal to that of Firmicutes and Bacteroidetes (Middelbos et al., 2010). This result corresponded with the findings of Suchodolski et al. (2008) who reported codominance of these 3 phyla in the colon of dogs based on sequence data generated by the Sanger method. Using a metagenomic approach in the same dogs used by Middelbos et al. (2010), Swanson et al. (2010) observed a bacterial codominance of Bacteroidetes (37 to 38%) and Firmicutes (30 to 35%) as reported by Turnbaugh et al. (2007), but a much smaller Fusobacteria population. It is likely that methodology was an important factor affecting the results of these studies and, thus, must be considered in the future.

Interestingly, Middelbos et al. (2010) observed a diet-specific alteration in the prevalence of fecal microbial phyla. Feeding dietary fiber (i.e., 7.5% beet pulp) to dogs increased sequence percentage of Firmicutes (15 to 28%), but decreased sequence percentage of Fusobacteria (40 to 24%) and Actinobacteria (1.4 to 0.8%), with little changes in Bacteroidetes. Likewise, Barry (2010) found that different sources of dietary fiber (i.e., cellulose, pectin, and fructooligosaccharides) modified the composition of bacterial phyla in the feces of cats. For instance, cats fed the diet containing 4% pectin had a greater percentage of Firmicutes and Spirochaetes than cats fed the diet containing 4% cellulose or 4% fructooligosaccharides. Moreover, the addition of dietary fructooligosaccharides increased the sequence percentage of Actinobacteria. According to metagenomic analyses, Barry (2010) also observed differences in microbial gene content associated with the metabolism of carbohydrates, AA, N, and aromatic compounds among dietary treatments. The analysis of carbohydrate-active enzyme showed significant alterations in glycoside hydrolases, glycosyl transferases, and carbohydrate-binding molecules when cats were fed the diet containing pectin or fructooligosaccharides.

Others have used such techniques to study antibiotic treatment. For example, Suchodolski et al. (2009) used pyrosequencing methods in the small intestine of dogs to identify the presence of 10 phyla in the jejunum. In that study, it was determined that Proteobacteria was the most dominant (47%) phyla and Firmicutes the second most dominant (15%), with the prevalence of these bacterial phyla being altered by tylosin administration. The rapid evolution of the pyrosequencing technique and similar emerging techniques and their incorporation into microbial studies will have a great impact on future research related to the health of dogs and cats. To our knowledge, such techniques have not been applied to the oral cavity, skin, or urogenital tract of dogs or cats, but these are areas that deserve more attention in the future.

Because measuring only the presence of genes (i.e., DNA content) does not provide information as to gene activity/expression, the measure of microbial mRNA and metabolites is required. Transcriptomics and metabolomics techniques currently used to study host physiology may be applied to microbial communities for a more in-depth characterization and measure of functional capacity. Although such techniques are not described here, they will be increasingly important in the future for an improved understanding of microbial ecosystems and how they affect the host.

**SUMMARY AND CONCLUSION**

Diverse and complex microbial communities exist in various body regions of canines and felines. These microbial communities are now appreciated as being a metabolically active organ of their own, with the potential to greatly influence host physiology and phenotype. Although the majority of microbes cannot be cultured using traditional methods, new molecular technologies have allowed the characterization of microbial communities without culture and in much greater specificity and depth. An improved understanding of microbial phylology and metabolism will aid in studying host physiology and pathology. As compared with humans, rodents, and livestock, much less attention has been paid to microbial composition and its dynamics in dogs and cats. Initial high-throughput sequencing studies have provided the general characteristics of intestinal microbial ecology. It is now time to focus on other body regions of dogs and cats. Such research will provide a better understanding of the physiological role of microbes in canine and feline health and disease, and help identify nutritional or pharmaceutical treatment strategies.


