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Current state of knowledge: the canine gastrointestinal microbiome

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Abstract

Gastrointestinal (GI) microbes have important roles in the nutritional, immunological, and physiologic processes of the host. Traditional cultivation techniques have revealed bacterial density ranges from 10^4 to 10^5 colony forming units (CFU)/g in the stomach, from 10^5 to 10^7 CFU/g in the small intestine, and from 10^9 to 10^{11} CFU/g in the colon of healthy dogs. As a small number of bacterial species can be grown and studied in culture, however, progress was limited until the recent emergence of DNA-based techniques. In recent years, DNA sequencing technology and bioinformatics have allowed for better phylogenetic and functional/metabolic characterization of the canine gut microbiome. Predominant phyla include Firmicutes, Bacteroidetes, Fusobacteria, Proteobacteria, and Actinobacteria. Studies using 16S ribosomal RNA (rRNA) gene pyrosequencing have demonstrated spatial differences along the GI tract and among microbes adhered to the GI mucosa compared to those in intestinal contents or feces. Similar to humans, GI microbiome dysbiosis is common in canine GI diseases such as chronic diarrhea and inflammatory bowel diseases. DNA-based assays have also identified key pathogens contributing to such conditions, including various *Clostridium*, *Campylobacter*, *Salmonella*, and *Escherichia* spp. Moreover, nutritionists have applied DNA-based techniques to study the effects of dietary interventions such as dietary fiber, prebiotics, and probiotics on the canine GI microbiome and associated health indices. Despite recent advances in the field, the canine GI microbiome is far from being fully characterized and a deeper characterization of the phylogenetic and functional/metabolic capacity of the GI microbiome in health and disease is needed. This paper provides an overview of recent studies performed to characterize the canine GI microbiome.

Keywords: diet, gastrointestinal health, pathogens

Introduction

The symbiotic relationship that exists between GI microbes and the host is critical for proper function of nutritional, developmental, immunological, and physiologic processes in animals, and thus contributes to host health (Mackie *et al.*, 1999; Hooper *et al.*, 2001). The resident microbiota forms an integral part of the intestinal barrier, which protects the host from pathogens. This protective mechanism is known as colonization resistance and includes competition for nutrients and mucosal adhesion sites, which creates a physiologically restrictive environment for invading pathogens (Kanauchi *et al.*, 2005). Furthermore, GI microbes contain enzymes that digest complex carbohydrates from the diet that are not digested by host enzymes and ferment endogenous products such as sloughed epithelial cells and mucus. The primary end-products of the fermentation process, the short-chain fatty acids (SCFA), namely acetate, propionate, and butyrate, provide energy for host epithelial cell growth and metabolism (Sunvold *et al.*, 1995). SCFA

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also have immuno-modulatory properties and modulate colonic and intracellular pH, both of which indirectly alter the composition of gut microbes (e.g., reduces the growth and activity of potential pathogens) and have application to GI inflammatory diseases (Vernia *et al.*, 2003). Decreased GI pH also results in decreased solubility of bile acids, thus decreased reabsorption and enterohepatic circulation of bile acids, increased absorption of minerals, and reduced absorption of ammonia (Vince *et al.*, 1978; Metzler-Zebeli *et al.*, 2010), all of which may have implications for the nutritional status of the host and fermentation that occurs in the hindgut.

Although microbial populations are present in many areas of the body, including the oral cavity, urinary and genital tracts, and skin, the GI tract harbors the largest microbial population. The bacterial density ranges from 10^4 to 10^5 CFU/g in the stomach to 10^5 to 10^7 CFU/g in the small intestine to 10^9 to 10^{11} CFU/g in the colon (Benno *et al.*, 1992; Johnston, 1999; Mentula *et al.*, 2005). Various host factors, such as genetic background, age, sex, initial environmental exposure and diet contribute to the development and maintenance of the core intestinal microbiota (Louis *et al.*, 2007; Khachatryan *et al.*, 2008; Mariat *et al.*, 2009). Although the upper GI tract harbors many microbes, the colon has the richest microbial population, most of which are anaerobes, and is the main site of the anaerobic digestion process known as fermentation. These microbes metabolize and survive on substrates from the host's diet (e.g., non-digestible carbohydrates and; undigested proteins) and endogenous secretions (Rowland *et al.*, 1985). Despite the dog's evolution in the order Carnivora and a lower consumption of non-digestible carbohydrates than most domestic livestock species, commercial dog foods may contain ingredients that significantly affect the population and/or activity of GI microbes and the fermentation process. Even when dogs are fed high-fiber diets, they do not rely heavily on the GI microbiota to meet their daily metabolizable energy requirement. A balanced microbial population, however, is very important for overall host health.

The emergence of metabolic and intestinal dysbiosis in humans has generated interest in gut microbes in recent years, as gut microbiome changes have been associated not only with colon cancer (Flint *et al.*, 2007) and inflammatory bowel disease (IBD; Friswell *et al.*, 2010) but also with complex metabolic diseases such as obesity (Ley *et al.*, 2005; Turnbaugh *et al.*, 2008) and diabetes (Wen *et al.*, 2008). Similarly in dogs, the intestinal microbiota has been associated with IBD (Suchodolski *et al.*, 2010) and chronic diarrhea (Bell *et al.*, 2008; Jia *et al.*, 2010). Recent advances in DNA sequencing technology and application of a variety of 'omic' technologies in a systems biology framework, have allowed for a better phylogenetic characterization of the gut microbiome. Furthermore, the metagenomics approach has improved our understanding of microbial function in the GI tract. A deeper understanding of the gut microbiome will provide reference values for healthy populations and assist in diagnosing and treating diseased animals. In addition, dietary interventions to prevent dysbiosis and thus maintain gut health in companion animals may be achieved.

Canine gut microbiome in the pre-sequencing era

Stomach and small intestine

The physiologic changes that occur throughout the GI tract, including the acidic nature of the stomach, and the bile salts and enzymes present in the small intestine, have an effect on what species of microbiota inhabit each region. Traditional studies reported that a mixture of aerobes and anaerobes inhabited the stomach, dominated by Gram-positive bacteria (Greene, 1998). Similar to humans, the Gram-negative *Helicobacter* spp. (e.g., *Helicobater felis*, *Helicobater Bizzozeronii*, and *Helicobater salomonis*) are also present and considered to be potential pathogens in the canine stomach (Fox, 1998), although their pathological role is not clear (Neiger and Simpson, 2000). *Eubacterium*, *Bacteroides*, *Clostridium*, *Fusobacterium*, *Bifidobacterium*, and *Lactobacillus* spp. are predominant in the canine duodenum and jejunum (Hermanns *et al.*, 1995; Johnston, 1999). The published results generated using bacterial cultivation techniques revealed that *Bacteroides*, *Eubacterium*, *Fusobacterium*, *Clostridium*, *Lactobacillus*, and *Bifidobacterium* are the most common anaerobic bacteria in the canine ileum (Davis *et al.*, 1977; Benno *et al.*, 1992). Likewise, *Streptococcus*, *Staphylococcus*, *Pasteurella*, *Escherichia*, and *Enterobacter* are the most common aerobic and facultative anaerobic bacteria in the canine ileum. Potential pathogenic species present in the small intestine include *Clostridium perfringens*, *Clostridium difficile*, *Klebsiella pneumoniae*, *Campylobacter jejuni*, *Salmonella* Typhimurium, enteric *Helicobacter* spp., *Yersinia enterocolitica*, and enteropathogenic *Escherichia coli* (Marks and Kather, 2003).

Large intestine

The large intestine has the highest density and diversity of bacteria (10^9–10^{10} CFU/g of content; Greene, 1998). In 1977, 84 species within 27 genera were cultivated from ileal, cecal, and colonic contents of dogs (Davis *et al.*, 1977). Although many of the microbes present in the large intestine were also detected in the small intestine, their abundance was much greater in the large intestine. Predominant genera included *Bacteroides*, *Bifidobacterium*, *Fusobacterium*, *Peptostreptococcus*, *Eubacterium, Clostridium, Peptococcus*, and *Lactobacillus* (Davis *et al.*, 1977). Similarly, the prevalence of potential pathogenic bacteria such as *C. perfringens*, and *E. coli*

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Table 1. Animals, experimental design, and analytical methods used in selected studies aimed at characterizing the canine GI microbiome

Reference	Sample type	Type of dog	Age of dog	Breed	Sex	BCS	Method
Garcia-Mazcorro *et al.* (2012)	Gastric and duodenal biopsies	Research dogs (*n*=8)	0.75 years	Mixed breeds	Four females, four males	–	qPCR, FISH, V1–V3 region 16S rRNA gene pyrosequencing
Garcia-Mazcorro *et al.* (2011)	Fecal	Privately owned dogs (*n*=12)	0.7–10.2 years	Ten different breeds	Five females, seven males	4–8 (9 point scale)	V1–V3 region 16S rRNA gene pyrosequencing
Handl *et al.* (2011)	Fecal	Privately owned dogs (*n*=12)	0.7–10.2 years	Ten different breeds	Five females, seven males	4–8 (9 point scale)	V1–V3 region 16S rRNA gene pyrosequencing
Swanson *et al.* (2011)	Fecal	Research dogs (*n*=6)	1.7 years	Two mongrels, four hound-crosses	Females	No obese dogs	Whole genome pyrosequencing
Middelbos *et al.* (2010)	Fecal	Research dogs (*n*=6)	1.7 years	Two mongrels, four hound-crosses	Females	No obese dogs	V3 region 16S rRNA gene pyrosequencing
Jia *et al.* (2010)	Fecal	Research dogs (*n*=8)	4–13 years	Beagles	–		FISH hybridization
Suchodolski *et al.* (2009)	Jejunal mucosa	Research dogs (*n*=5)	2 years	–	–	3–4 (5-point scale)	16S rRNA gene sequencing
Xenoulis *et al.* (2008)	Duodenal biopsies	Privately owned dogs (*n*=9)	2.7–6 years	Three greyhounds, six beagles	Six females, three males	–	16S rRNA gene pyrosequencing
Suchodolski *et al.* (2008)	Duodenum, jejunum, ileum, and colon	Research dogs (*n*=6)	3.6–7 years	Hound dogs	Three males, three females	–	V1–V3 region 16S rRNA gene pyrosequencing

were also reported in the large intestine (Davis *et al.*, 1977). Prior to the 21st century, most of the current knowledge about canine GI microbiota was generated using cultivation assays. In addition to being very laborious, time consuming, and requiring immediate processing, cultivation techniques do not allow for identification and differentiation of most microbial strains. As there is a lack of information regarding the growth requirements of most microbes, only a minor fraction of GI microbes have been isolated and studied in culture to date (Eckburg *et al.*, 2005).

Canine gut microbiome in the sequencing era

To overcome the limitations of culture, molecular-based techniques have been used to study GI microbial ecology over the past decade. The primary target for molecular approaches is the 16S ribosomal RNA (rRNA) gene because of its presence in all microbes and because it contains both conserved and variable sequence regions across species (Clarridge, 2004). Molecular fingerprinting techniques, including denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), and terminal restriction fragment length polymorphism (T-RFLP), were initially used to study qualitative differences in GI microbial ecology. For identification, DNA bands of interest may be isolated and sequenced. These techniques are rather insensitive, however, and provide information on only the most predominant bacterial groups. Quantitative PCR (qPCR) and fluorescent *in situ* hybridization (FISH), which are more sensitive than the gel-based techniques, have been widely used over the last decade to quantify select bacterial groups. The primary issue with qPCR and FISH is that they are limited in scope, only providing data on a few specific microbial groups.

Recently, DNA-sequencing techniques that use automated high-throughput sequencing platforms have increased our knowledge regarding GI microbial identification and classification. These techniques are being used in the human microbiome projects to characterize the entire microbial community present in the GI tract, mouth, vagina, nasal cavity, skin and other body regions (Peterson *et al.*, 2009). These high-throughput DNA-sequencing techniques have recently been applied to describe the phylogenetic structure and functional capacity of the canine GI microbiome. Details pertaining to study design, dog number and characteristics (e.g., age, breed, sex and body condition score), and method of analyses used in these experiments are summarized in Table 1. Although the samples studied have included some mucosa and digesta samples from various segments of the GI tract, most of the studies have focused on bacteria in fecal samples as a proxy for gut bacteria. As bacterial populations are known to differ between intestinal biopsy and fecal samples (Momozawa *et al.*, 2011);

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however, data from fecal samples may not be completely representative of data in the more proximal parts of the intestine.

Stomach of healthy dogs

Garcio-Mazcorro *et al.* (2012) used FISH, qPCR, 16S rRNA gene libraries, and 454 pyrosequencing of 16S rRNA gene amplicons (V1–V3 region) to study the microbiome of the canine stomach. Gastric biopsies were collected from eight clinically healthy mixed-breed research dogs. qPCR results indicated 10^5 16S rRNA gene copies of total bacteria present, with a predominance of *Helicobacter* and *Lactobacillus* spp. The total sequence number obtained by pyrosequencing from the stomach was 142,026 and ranged from 1199 to 7734 per sample. A median of 36 operational taxonomic units (OTUs) (range of 18–119) were detected per dog. The majority of the sequences belonged to Proteobacteria (99.6%), with only a few Firmicutes (0.3%). The Proteobacteria included the genus *Helicobacter* (98.6%) in gastric biopsies (Garcia-Mazcorro *et al.*, 2012).

Duodenum of healthy dogs

The microbial communities of canine duodenal mucosa and contents were recently studied using 16S rRNA gene clone libraries and 454 pyrosequencing (Suchodolski *et al.*, 2008; Xenoulis *et al.*, 2008; Garcia-Mazcorro *et al.*, 2012). The microbial community of duodenal biopsy samples of healthy client-owned dogs was represented by six primary phyla: Firmicutes (46.4%), Proteobacteria (26.6%), Bacteroidetes (11.2%), Spirochaetes (10.3%), Fusobacteria (3.6%), and Actinobacteria (1.0%) (Xenoulis *et al.*, 2008). Similarly, Garcia-Mazcorro *et al.* (2012) reported the predominance of Firmicutes (46.9%), Proteobacteria (21.2%), and Bacteroidetes (6.9%), which comprised more than 80% of all sequences in duodenal biopsies of healthy research dogs. Within Firmicutes, the orders Clostridiales (19.6%), Lactobacillales (14.1%), and Bacillales (12%) and families Clostridiaceae (13.9%), Carnobacteriaceae (7.4%), and Turicibacteriaceae (8.9%), were most abundant in duodenal mucosa samples (Xenoulis *et al.*, 2008). The family Helicobacteriaceae was the most abundant family within Proteobacteria (13.2%), followed by Pasteurellaceae (6.5%) and Moraxellaceae (1.9%). Bacteroidetes (11.2%) and Spirochaetes (10.2%) were two other phyla present in the duodenal mucosa, represented by the families Porphyromonadaceae (7.2%) and Spirochaetaceae (10.3%), respectively (Xenoulis *et al.*, 2008).

Suchodolski *et al.* (2008) examined the bacterial microbial communities of duodenal, jejunal, ileal, and colonic digesta of healthy research dogs. The bacterial diversity indices increased gradually along the GI tract from the duodenum to the colon. Similar to mucosal samples, duodenal digesta samples were dominated by phyla Firmicutes and orders Clostridiales (~40%) and Lactobacillales (~22%) (Suchodolski *et al.*, 2008). The distribution of two predominant *Clostridium* clusters (XI and XIVa) was represented by 25% and 31% of the duodenal digesta samples, respectively. Proteobacteria (32%), including *E. coli*-like organisms, was also abundant in duodenal digesta of these dogs. Anaerobic Fusobacteriales and Bacteroidales were only present sporadically in duodenal digesta (Suchodolski *et al.*, 2008).

Jejunum of healthy dogs

A detailed analysis of the microbiome inhabiting the jejunal mucosa of healthy research dogs was conducted by Suchodolski *et al.* (2009) using 16S rRNA gene pyrosequencing. The predominant bacterial phyla measured were Proteobacteria (46.7%), Firmicutes (15.0%), Actinobacteria (11.2%), Spirochaetes (14.2%), Bacteroidetes (6.2%), and Fusobacteria (5.4%). That study also identified four additional phyla that were not reported in dogs previously: Tenericutes, Cyanobacteria, Verrucomicrobia, and Chloroflexi, which were all present in low abundance (<0.1%). The most abundant bacterial classes in jejunal mucosa samples were Gamma-proteobacteria (~40%), followed by Spirochaetes (~13%), Actinobacteria (~12%), Clostridia (~10%), Bacteroidetes (~6%), Fusobacteria (~4%), and Alpha-proteobacteria (~3%). Predominant bacterial families included Moraxellaceae (33.7%), Spirochaetaceae (14.1%), Corynebacteriaceae (7.0%), Clostridiaceae (5.5%), Enterobacteriaceae (4.6%), and Fusobacteriaceae (5.4%).

Suchodolski *et al.* (2008) used 16S rRNA gene clone sequencing to examine the microbial communities of canine jejunal digesta contents in healthy research dogs. Firmicutes was the most abundant phylum, with Clostridiales (~40%), Lactobacillales (10%), and Enterobaceriales (~30%) being the most abundant bacterial orders. Fusobacteriales was also present at ~10% and at greater concentrations than in duodenal digesta samples (~3%) of these dogs. *Clostridium* cluster XI was present in greater concentrations (51%) than cluster XIVa (11%), with *Clostridium hiranonis*, *Clostridium glycolicum*, and *Clostridium propionicum* being the predominant species.

Ileum of healthy dogs

The ileal digesta contents collected from healthy research dogs are dominated by Fusobacteria, Firmicutes, and Bacteroidetes (Suchodolski *et al.*, 2008), with the orders Fusobacteriales (~30%), Clostridiales (25%), Bacteroidales (22%), and Enterobacteriales (~20%) predominating (Suchodolski *et al.*, 2008). Both *Clostridium* clusters XI and XIVa were present in ileal digesta, but at low

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Table 2. Bacterial phyla (expressed as percentage of sequences) in fecal and mucosal samples of select canine GI microbiome studies

Reference	Sample type	Method	Actinobacteria	Bacteroidetes	Firmicutes	Fusobacteria	Proteobacteria
Garcia-Mazcorro *et al.* (2011)	Fecal	V1–V3 region 16S rRNA gene pyrosequencing	0.9–2.0	0.1–1.1	97.5	0.1–0.8	0.1
Handl *et al.* (2011)	Fecal	V1–V3 region 16S rRNA gene pyrosequencing	1.8	2.2	95	0.3	–
Swanson *et al.* (2011)	Fecal	Whole genome pyrosequencing	1	37–38	31–35	7–9	13–15
Middelbos *et al.* (2010)	Fecal	V3 region 16S rRNA gene pyrosequencing	0.8–1.4	32–34	15–28	24–40	5–6
Suchodolski *et al.* (2009)	Jejunal mucosa samples	16S rRNA gene pyrosequencing	11.2	6.2	15	5.4	46.7
Xenoulis *et al.* (2008)	Duodenal biopsies	16S rRNA gene sequencing	1.0	11.2	46.4	3.6	26.6
Suchodolski *et al.* (2008)	Duodenum, jejunum, ileum and colon contents	V1–V3 region 16S rRNA gene pyrosequencing	–	12.4	47.7	16.6	23.3

abundance (8–18%). In contrast to digesta sampled from the duodenum and jejunum, ileal contents had much lower abundance of *Lactobacillus* spp. (1.4%).

Colon of healthy dogs

Colon contents of healthy research dogs were assessed using 16S rRNA gene clone sequencing and were co-dominated by Fusobacteria, Bacteroidetes, and Firmicutes (Suchodolski *et al.*, 2008). Fusobacteriales (∼30%) and Bacteroidales (∼30%) were the most abundant bacterial orders in the colonic contents of healthy dogs. The presence of Fusobacteria (10^8 CFU per ml of intestinal content) also has been demonstrated using culture-based techniques (Davis *et al.*, 1977). The next abundant order present was Clostridiales (∼18%), with *Clostridium* cluster XIVa being the predominant member (50%). The presence of *Clostridium* cluster XIVa was likely due to functional and metabolic capacity of the members of this group. This cluster includes *Eubacterium*, *Roseburia*, and *Ruminococcus* groups that are dietary fiber fermenters. Proteobacteria, including *E. coli*-like organisms, appeared to be present at low concentrations (1.4%) in the digesta of the canine colon. Lactobacillales in colonic digesta (∼10%) were higher than in ileal digesta (∼1.4%) (Suchodolski *et al.*, 2008). Studies using FISH indicated the presence of mucosa-associated bacteria in the healthy colon, mainly on the outer mucous layer and not adherent to epithelial cells (Simpson *et al.*, 2006).

Feces of healthy dogs

FISH was performed on fecal samples of eight healthy beagle dogs between 4 and 13 years of age (Jia *et al.*, 2010). The most abundant phylum present in healthy dog feces was Firmicutes (31%) and included *Clostridium*, *Eubacterium*, and the *Lactobacillus–Enterococcus* group. Actinobacteria (12%) was another predominant phylum in the fecal samples of healthy dogs, with the presence of the *Atobium* cluster and bifidobacteria (10^8 cells/g of feces). In the same study, Bacteroidetes was present at about 10^8 cells/g of feces and consisted of <1% of total bacteria in the feces of dogs. Middelbos *et al.* (2010) was the first to use 16S rRNA gene pyrosequencing to study fecal microbial communities of healthy adult research dogs. Sequencing of the V3 hypervariable region resulted in 17,828 high-quality sequences from five individual fecal samples of dogs fed a control diet and 21,660 sequences from five individual fecal samples of dogs fed a beet pulp-containing diet (7.5% of diet). The results indicated the predominance of the phyla Firmicutes (15–28%), Bacteroidetes (32–34%), Fusobacteria (24–40%), Actinobacteria (0.8–1.4%), and Proteobacteria (5–6%) (Table 2). Although Firmicutes and Bacteroidetes have been reported to co-dominate the human fecal

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Table 3. Most abundant bacterial families and genera in fecal and mucosal samples of select canine GI microbiome studies

Reference	Sample type	Method	Most abundant families	Most abundant genera
Handl *et al.* (2011)	Fecal	V1–V3 region 16S rRNA gene pyrosequencing	Ruminococcaceae, Clostridiaceae, Lachnospiracea, and Erysipelotrichaceae	*Clostridium, Ruminococcus, Dorea, Roseburia, Clostridium* clusters XIVa and cluster XI,
Swanson *et al.* (2011)	Fecal	Whole genome pyrosequencing	–	–
Middelbos *et al.* (2010)	Fecal	V3 region 16S rRNA gene pyrosequencing	–	–
Suchodolski *et al.* (2009)	Jejunal mucosa samples	16S rRNA gene pyrosequencing	Moraxellaceae, Spirochaetaceae, Corynebacteriaceae, Clostridiaceae, Enterobacteriaceae, and Fusobacteriaceae	–
Xenoulis *et al.* (2008)	Duodenal biopsies	16S rRNA gene pyrosequencing	Clostridiaceae, Carnobacteriaceae, Turicibacteriaceae, Pasterellaceae, Helicobacteriaceae, Porphyromonadaceae, and Spirochaetaceae	–
Suchodolski *et al.* (2008)	Duodenum, jejunum, ileum and colon contents	V1–V3 region 16S rRNA gene sequencing	–	*Fusobacterium, Lactobacillus, Streptococcus, Clostridium, Escherichia,* and *Klebsiella*

–, Data not provided.

microbiome that study suggested that canine feces were co-dominated by Firmicutes, Bacteroidetes, and Fusobacteria. Fecal samples from this study were also analyzed using shotgun sequencing to assess the functional and metabolic capacity of the canine fecal microbiome in addition to its phylogenetic composition (Swanson *et al.*, 2011). Shotgun sequencing identified the same phyla; however, the abundance of Bacteroidetes and Firmicutes (~35%) was much higher than that of Fusobacteria (7–8%), suggesting a bias involved with the generation of amplicons for the 16S rRNA gene-based method. Within Firmicutes, the class Clostridia was predominant (83–90%).

Two recent studies (Handl *et al.*, 2011; Garcia-Mazcorro *et al.*, 2011) examined fresh fecal samples collected from 12 client-owned dogs of a wide age range (0.7–10.2 years) and of 10 breeds. These studies used V1–V3-specific primers to generate 16S rRNA amplicons for pyrosequencing (Table 1). Both studies reported the predominance of Firmicutes (95–97.5%) (Table 2), comprising, the classes Clostridia, Bacilli, and Erysipelotrichi (Table 2). Ruminococcaceae, Clostridiaceae, Lachnospiraceae, and Erysipelotrichaceae were the most abundant families present in the fecal samples of these dogs (Table 3). The clostridia class was dominated by the *Clostridium* (22.7%) and *Ruminococcus* (17.4%) genera. *Dorea* (7%) and *Roseburia* (5%) were the other prevalent genera reported. Within *Clostridium*, clusters XIVa (~60% of all Clostridiales sequences) and cluster XI (~34%) were most abundant. Both clusters have been linked to GI health outcomes, as members of both clusters actively produce SCFA, especially butyrate. Butyrate is the preferred energy source of colonocytes and is important for GI health via stimulation of cell proliferation, promotion of apoptosis, and prevention of colon cancer (Wong *et al.*, 2006). In contrast to the results of Middelbos *et al.* (2010), these two studies reported a very low abundance of Bacteroidetes (2.2%), and its predominant genera *Prevotella* (0.04%) and *Bacteroides* (0.022%). The abundance of Actinobacteria was also quite low (1.8%). Coriobacteriaceae, composed of the genera *Collinsella* and *Slackia*, was the main family present within Actinobacteria. In contrast to Middelbos *et al.* (2010), Fusobacteria were reported to be present at very low abundance (0.3%) in that study. Although a more in-depth evaluation of the canine GI microbiota is needed, these initial studies highlight the spatial differences that exist among the various sections of intestine and fecal samples.

Although 16S rRNA gene pyrosequencing has many advantages over traditional culture, its limitations cannot be ignored. Differences in DNA extraction methods and PCR primers may result in the over- or under-representation of some microbial groups. The focus on the 16S rRNA gene also only provides phylogenetic information. Thus, other techniques are needed to understand microbial metabolism and host–microbe interactions. A metagenomics approach that uses shotgun

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sequencing provides phylogenetic and functional information on the microbiome. Using 1,008,341 DNA sequences from two pooled samples from healthy adult research dog feces, Swanson *et al.* (2011) reported the gene content of the canine microbiome. Primary functional categories were not significantly affected by diet and were associated with carbohydrates (12.5–13.0% of sequences); protein metabolism (8.1–9.1%); DNA metabolism (7.1–7.4%); cell wall and capsule (7.0–7.6%); amino acids and derivatives (6.8–6.9%); virulence (6.2–7.2%); and cofactors, vitamins, prosthetic groups and pigments (5.7–6.0%).

Effects of dietary intervention on canine GI microbiota

Many commercial dog foods contain ingredients that significantly affect the population and/or activity of GI microbes and the fermentation process. Similar to what is done with human foods, dietary fiber, prebiotics, and probiotics are often added to canine diets as a means to enhance GI health. The notion that commensal GI microbes are beneficial is not new. In fact, the modulation of GI microbes by probiotics was established early in the 20th century. Manipulation of canine GI microbiota to improve health via dietary intervention, however, did not begin until the early 1990s. Many studies have indicated the beneficial effects of feeding dietary non-digestible carbohydrates, including resistant starch, non-starch polysaccharides, and non-digestible oligosaccharides, to dogs. At the proper concentrations, non-digestible carbohydrates can improve laxation and stool quality, and alter microbial populations and fermentative end-product concentrations. Dietary fiber and prebiotics have been the major nutritional strategies studied to modulate the canine GI microbiota. Fructans, including chicory root extract, inulin, oligofructose, and short-chain fructooligosaccharides (scFOS) have been studied in greater detail in dogs than other ingredients with prebiotic potential. Other ingredients used in this regard include yeast cell wall (YCW) extracts, galactooligosaccharides, lactosucrose, lactulose, maltodextrin-like oligosaccharides, transgalactooligosaccharides and xylooligosaccharides, pullulan, and γ-cyclodextrins.

Very few clinical experiments have been performed to test the ability of dietary fiber and prebiotics to promote GI health in dogs. More commonly, an array of GI health indicators, including fecal scores, pH, microbiota, and fermentation end-products, have been used to evaluate prebiotic efficacy in healthy dog populations. Due to their relationship with GI health, increased fecal bifidobacteria, lactobacilli, and butyrate are traditionally considered beneficial, while increased *C. difficile* or *C. perfringens*, *E. coli*, and protein catabolites (e.g., ammonia, phenols, indoles, biogenic amines, and volatile sulfur-containing compounds) are traditionally considered detrimental. As only a brief overview of this area is provided below, the authors refer to Swanson and Fahey (2006) and Vester and Fahey (2010) for an in-depth description of these prebiotic-based studies in dogs.

Fecal bifidobacteria and lactobacilli populations have long been used in human and animal studies as a gauge of prebiotic potential and GI health. Several forms of fructans, the most studied prebiotic class in dogs, have been reported to have prebiotic activity. Russell *et al.* (1998) conducted three experiments to study the effects of scFOS or chicory on fecal microbiota populations. Compared to the control, dogs fed scFOS (1%) or chicory (3%) had greater fecal bifidobacteria (1×10^9 CFU/g versus 8.2×10^{10} CFU/g versus 4.5×10^{10} CFU/g, respectively) and lower fecal clostridia (1.6×10^{11} CFU/g versus 1.3×10^{10} CFU/g versus 4.2×10^{10} CFU/g, respectively) concentrations in the first experiment. A second and a third experiment that evaluated increasing chicory (1.5, 3.0, and 5.0%) and chicory (0, 0.5, 2.0, and 4.0%) and soy (0, 6.0, and 12.0%) concentrations demonstrated similar effects. Beynen *et al.* (2002) reported greater fecal bifidobacteria in dogs fed 1% oligofructose compare to control (8.8×10^{10} versus 7.8×10^{10} CFU/g), while Swanson *et al.* (2002a) demonstrated similar effects in healthy adult dogs fed 4 g scFOS (~1.3% of food intake)+2 g YCW extract (~0.67% of food intake) per day. In that study, not only were fecal bifidobacteria greater (9.4×10^{10} versus 10.0×10^{10} CFU/g), but ileal lactobacilli (7.6×10^{10} versus 8.7×10^{10} CFU/g) and fecal lactobacilli (8.2×10^{10} versus 9.8×10^{10} CFU/g) were greater in dogs fed scFOS+YCW extract compared to controls. Swanson *et al.* (2002b, 2002c) demonstrated similar effects in healthy adult dogs fed 2 g (~0.5% of food intake) scFOS+2 g (~0.5% of food intake) YCW extract and 4 g (~0.8% of food intake) scFOS+2×10^9 CFU *Lactobacillus acidophilus* probiotic per day, respectively. In healthy geriatric dogs, consumption of 1% chicory or 1% YCW extracts resulted in greater fecal bifidobacteria compared to control dogs (10.1×10^{10} versus 10.5×10^{10} versus 10.6×10^{10} CFU/g) (Grieshop *et al.*, 2004). Blends of fermentable carbohydrates (beet pulp, FOS and YCW extract) have also been demonstrated to increase fecal bifidobacteria compared to a non-fermentable fiber (cellulose) control (Middelbos *et al.*, 2007).

The prebiotic potential of other carbohydrate-based ingredients has been tested in healthy dogs. For example, lactosucrose, a trisaccharide produced from lactose and sucrose, has been shown to increase bifidobacteria populations (8.9 CFU log 10/g at baseline versus 9.4 CFU log 10/g 14 days of supplementation; Terada *et al.*, 1992). In that study, lecithinase-positive clostridia populations, which include *C. perfringens*, were decreased after 14 days (3.1 versus 6.0 CFU log 10/g) of lactosucrose feeding. Inclusion of pullulan (4 g/day), a naturally occurring fermentation product produced by *Aureobasidium pullulans*, increased ileal bifidobacteria (~0.7 log unit increase) and lactobacilli (~0.9 log unit increase) populations in healthy adult research dogs (Spears *et al.*,

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2005). Finally, Faber *et al.* (2011) reported a quadratic increase in bifidobacteria after supplementation of increasing dietary galactoglucomannanoligosaccharide concentrations (0.0, 0.5, 1, 2, 4, and 8%). An approximate 2 log unit increase (those fed 8.0 versus 9.8 CFU log 10/g) was noted between dogs fed the control and 8% galactoglucomannanoligosaccharides.

Although the majority of prebiotic studies have focused on fecal samples of healthy adult dogs, Apanavicius *et al.* (2007) performed an experiment aimed at evaluating the protective effects of 1% scFOS or 1% inulin during a pathogen (*Salmonella* Typhimurium) challenge in weanling (12-week-old) puppies. Results indicated that infected puppies consuming fructans had a lower severity of enterocyte sloughing than those fed the control diet. Puppies fed inulin also had higher fecal acetate, total SCFA concentrations and lactobacilli. Thus, this study indicated that prebiotics such as inulin and FOS appeared to attenuate some of the negative effects of Salmonella challenge and may provide protection against infection in weanling puppies.

Although the above studies have provided evidence that prebiotics and fibers have the ability to manipulate the GI microbiota of dogs, the field as a whole has many limitations. First, most prebiotic studies have used traditional plating techniques or qPCR to quantify a limited number of bacteria (e.g., *Lactobacillus*, *Bifidobacteria*, *C. perfringens*, and *E. coli*) to form conclusions regarding prebiotic potential. Such studies do not provide a widespread phylogenic and taxonomic characterization of gut microbial communities. Second, most studies were performed in healthy adult research populations rather than the ill or susceptible (e.g., weanling and geriatrics) animals most likely to benefit from such dietary intervention. Third, most studies have analyzed fecal samples rather than mucosal or digesta samples within the GI tract, so application to disease is sometimes questioned. Lastly, a wide range of prebiotic dosages have been tested across the experiments, often making it difficult to compare one study to another.

Recent canine fiber and prebiotic experiments incorporated the use of 454 pyrosequencing to provide a more in-depth view of the GI microbial communities and how they may be impacted by diet. The first nutritional intervention study in dogs that used pyrosequencing was performed by Middelbos *et al.* (2010), with a goal to assess the effects of beet pulp fiber on fecal microbial composition. Dogs fed a control diet were compared to dogs fed a diet containing 7.5% beet pulp in a crossover design with 14 days periods. Beet pulp is a very common dietary fiber source in commercial dog foods and consists of a complex mixture of fermentable and non-fermentable carbohydrates. In that study, the abundance of Actinobacteria and Fusobacteria was lower when dogs were fed the beet pulp diet compared to control. Within Firmicutes, Clostridia abundance was greater in beet pulp-fed dogs compared to control dogs. *Eubacterium halii*, a butyrate producer, was only present in beet pulp-fed dogs. Furthermore, an overrepresentation of *Faecalibacterium prausnitzii* and an under-representation of *Fusobacteria* was observed when dogs were fed the beet pulp-containing diet. Similar to *E. halii*, *F. prausnitzii* is another butyrate producer and has been shown to possess anti-inflammatory properties.

The effects of a synbiotic formulation that included a probiotic (5×10^9 CFU of a mixture of seven bacterial species) and prebiotic (fructooligosaccharides, arabinogalactans) in dogs were reported recently (Garcia-Mazcorro *et al.*, 2011). Changes in fecal microbes were evaluated using 454 pyrosequencing. The synbiotic capsules were fed to privately owned dogs for 21 days. The most abundant phylum in feces of all dogs was Firmicutes, followed by Actinobacteria and Bacteroidetes, regardless of synbiotic treatment. Synbiotic treatment increased the abundance of family Eubacteriaceae. The proportion of the *Eubacterium* genus was greater after synbiotic treatment as compared to baseline values. Similarly, the proportion of phylum Fusobacteria and all phylogenetic levels down to the genus *Fusobacterium* were significantly higher during synbiotic administration. Massive parallel 454 pyrosequencing is a great tool that allows the characterization of bacterial groups at various taxonomic levels. More research using this technology is needed to expand our knowledge of GI tract microbial communities in healthy versus diseased dog populations and to study the effects of dietary intervention.

Effects of disease on canine GI microbiota

The invasion and/or colonization of the GI tract with specific pathogens may profoundly disturb the integrity of the intestinal epithelial barrier (Viswanathan *et al.*, 2009). Several potential GI pathogens are recognized in dogs, including *C. difficile*, *C. perfringens, C. jejuni*, *Salmonella* spp., and *E. coli* (Marks *et al.*, 2011). However, most of those are recognized commensal in the canine GI tract and have been isolated at similar frequencies from dogs with signs of GI disease and clinically healthy dogs (Marks and Kather, 2003). Therefore, the cause–effect relationships between those organisms and GI disease need to be interpreted with caution.

Non-specific alterations of the GI microbiota have also been regarded as a pivotal factor for the development of GI disease. As mentioned above, the balanced GI microbiota is responsible for crucial effects on the host. Therefore, a disturbed GI microbiota may result in intestinal disorders (e.g., bacterial deconjugation of bile acids may lead to malabsorption of fat and lipid-soluble vitamins; Hall, 2011). Small intestinal bacterial overgrowth (SIBO) and IBD are associated with a disturbed GI microbiota in humans. In dogs, the use of the term SIBO (typically $>10^5$ CFU/ml small intestinal content) is under debate, since a study has shown that much higher

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bacterial counts are commonly found in clinically healthy dogs (German *et al.*, 2003). Therefore, the alternative terms antibiotic-responsive diarrhea or small intestinal dysbiosis are currently used for dogs with this syndrome.

Recent studies have attempted to characterize the fecal microbiota of diarrheic dogs. Bell *et al.* (2008) assessed changes in the GI microbiota associated with acute episodes of diarrhea by using T-RFLP and qPCR, and observed large-scale changes of the GI microbiota during episodes of diarrhea. These included increased abundance of *Clostridiales* (especially *C. perfringens*) and *Enterococcus* spp. Jia *et al.* (2010) utilized FISH and DGGE to investigate the composition of fecal microbiota in dogs with chronic diarrhea. FISH analysis revealed a significantly higher *Bacteroides* count in dogs with chronic diarrhea compared to control dogs. The proportion of the *Lactobacillus–Enterococcus* group was 8% higher, albeit this was not significant, in control dogs than in dogs with chronic diarrhea. *Bifidobacteria* were not significantly different between the two groups (Jia *et al.*, 2010).

IBD is a common chronic GI disorder in dogs. Similarly to humans, interplay between the intestinal microbiota together with an underlying genetic susceptibility of the host and dietary and environmental factors, are implicated in the development of IBD (Virgin and Todd, 2011). Technological advances have allowed a more detailed understanding of the composition of the intestinal microbiota in IBD. In human IBD, lower abundance of *Firmicutes* and *Bacteroides*, and higher abundance of *Proteobacteria* compared to healthy subjects have been observed (Seksik, 2010). Similar changes in the small intestinal microbiota have been observed in dogs with IBD. Xenoulis *et al.* (2008) constructed 16S rRNA gene clone libraries from duodenal brush samples from healthy dogs and dogs with IBD. In this study, a significant lower species richness and significantly higher proportion of *Enterobacteriaceae* were observed in dogs with IBD (Xenoulis *et al.*, 2008). Suchodolski *et al.* (2010) applied the same technique to duodenal biopsies from dogs with IBD. These dogs had a significantly higher abundance of *Proteobacteria* and a significantly lower abundance of *Clostridia* (Suchodolski *et al.*, 2010).

Future directions

Recent advances in sequencing technology and bioinformatics have enabled the molecular characterization of GI microbial populations in humans and animals. Although the human microbiome projects have the support of the National Institutes of Health and will lay a strong foundation of science relating to microbial ecology and host–microbe interactions, more work is desperately needed in the dog population. Despite the recent progress made through the use of high-throughput sequencing techniques, the phylogenetic structure and functional/metabolic capacity of the healthy canine GI microbiome is far from being fully characterized. Moreover, the impact that dog breed, age, diet, and other environmental factors has on microbial groups, and how and which microbes contribute to GI and host metabolic diseases, is far from understood.

In the future, a deeper characterization of the phylogenetic and functional/metabolic capacity of canine GI microbiome, including the mucosal and luminal populations that exist throughout the GI tract, is needed. Furthermore, to obtain a better understanding of how the microbial populations interact with the host in times of health and disease, the application of transcriptomic-, proteomic-, and metabolomic-based techniques to host and microbial samples, will be required. Although these techniques are only now being applied to human GI microbiology, the same is needed in canine GI health. As the use of any single 'omics' approach will not be sufficient to characterize and understand the complexity of the host–microbe relationships that exist in the gut, an integrated approach is the key to understanding the role of the microbiome in canine health and disease.

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