©Cambridge University Press 2012
ISSN 1466-2523

Animal Health Research Reviews **13**(1); 64–77
doi:10.1017/S1466252312000060

Feline gastrointestinal microbiota

Yasushi Minamoto[1], Seema Hooda[2], Kelly S. Swanson[2] and Jan S. Suchodolski[1]*
[1]*Gastrointestinal Laboratory, Texas A and M University, 4474 TAMU, College Station, TX 77843-4474, USA,*
[2]*Department of Animal Sciences, University of Illinois, Urbana, IL 61801, USA*

Received 2 March 2012; Accepted 30 April 2012

Abstract

The close relationship between gastrointestinal (GI) microbiota and its host has an impact on the health status of an animal that reaches beyond the GI tract. A balanced microbiome stimulates the immune system, aids in the competitive exclusion of transient pathogens and provides nutritional benefits to the host. With recent rapid advances in high-throughput sequencing technology, molecular approaches have become the routinely used tools for ecological studies of the feline microbiome, and have revealed a highly diverse and complex intestinal ecosystem in the feline GI tract. The major bacterial groups are similar to those found in other mammals, with *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* constituting more than 99% of intestinal microbiota. Several nutritional studies have demonstrated that the feline microbiota can be modulated by the amount of soluble fibers (i.e., prebiotics) and macronutrients (i.e., protein content) in the diet. Initial clinical studies have suggested the presence of a dysbiosis in feline inflammatory bowel disease (IBD). Recently, metagenomic approaches have attempted to characterize the microbial gene pool. However, more studies are needed to describe the phylogenetic and functional changes in the intestinal microbiome in disease states and in response to environmental and dietary modulations. This paper reviews recent studies cataloging the microbial phylotypes in the GI tract of cats.

Keywords: Composition, development, role, health, disease, molecular methods, enteropathogens.

Introduction

The intestinal microbiota is defined as the consortium of all micro-organisms (i.e., bacteria, fungi, protozoa and viruses) inhabiting the gastrointestinal (GI) tract. Molecular–phylogenetic studies have revealed that the intestinal microbiota of mammals is highly diverse, harboring several hundred to over a thousand bacterial phylotypes (Frank *et al.*, 2007; Handl *et al.*, 2011; Swanson *et al.*, 2011). The mammalian intestine harbors 10^{10}–10^{14} micro-organisms, approximately 10 times the number of host cells. The resident microbiota provides many health benefits to the host. For example, resident microbes are able to help fend off invading pathogens. They aid in digestive processes and harvest energy from the diet that can be utilized by the host, thereby providing nutritional support for enterocytes. Furthermore, the presence of enteral microbiota is an important trigger for the development and constant stimulation of the immune system.

Molecular approaches have improved our understanding of the composition, the dynamics and the functionality of the intestinal ecosystem in many mammalian species, including the cat (Eckburg *et al.*, 2005, Desai *et al.*, 2008; Ritchie *et al.*, 2008, 2010). Various studies in humans and other animal species have revealed how the microbiota is influenced by diet, antimicrobials, and is altered in chronic intestinal inflammation (Johnston *et al.*, 2000; Eckburg *et al.*, 2005; Dethlefsen *et al.*, 2008; Suchodolski *et al.*, 2009; Gronvold *et al.*, 2010). Molecular–phylogenetic information about the effect of these environmental factors on intestinal microbiota in cats is still limited; however, several recently published studies have characterized the impact of age, nutritional intervention and GI disease on feline gut

*Corresponding author. E-mail: jsuchodolski@cvm.tamu.edu

https://doi.org/10.1017/S1466252312000060 Published online by Cambridge University Press

microbiota consortia (Inness *et al*., 2007; Abecia *et al*., 2010; Barry *et al*., 2010; Jia *et al*., 2011a; Hart *et al*., 2012). This article will review previous work characterizing the GI microbiome of cats.

Role of the GI microbiota in cats

The GI microbiota has attracted investigators for decades due to its potential etiopathologic role in host health and disease. Many studies in humans and other animal species have suggested that various diseases are associated with alterations of the GI microbiota. Specific enteropathogens have been recognized in cats (e.g., *Campylobacter* spp. and *Salmonella*), yet because most of them are found in similar frequency in healthy animals, the cause–effect relations remain elusive (Queen *et al*., 2012). Chronic enteropathies, such as inflammatory bowel disease (IBD) have been associated with changes in the proportions of specific bacterial groups, especially *Enterobacteriaceae* and *Desulfovibrio* spp. in some studies (Inness *et al*., 2007; Janeczko *et al*., 2008). Conversely, some recent clinical studies suggest that the administration of specific bacterial strains or products intended to alter the intestinal microbiota (i.e., probiotics, prebiotics, or synbiotics) have the potential to improve the frequency and/or duration of diarrhea in a subset of cats with specific acute or chronic GI diseases (Bybee *et al*., 2011; Hart *et al*., 2012). While the cat is an obligate carnivore, most commercial feline diets contain moderate quantities of carbohydrates. Furthermore, soluble fiber sources added to the diet (i.e., prebiotics) have been associated with changes in fecal characteristics and metabolites in healthy cats that are hypothesized to improve GI health (Vester *et al*., 2009; Barry *et al*., 2010). However, the role of these dietary compounds in acute and chronic intestinal inflammation requires further research as limited information is available in clinical patients (Abecia *et al*., 2010). In humans and mouse models, a plethora of other extra-intestinal diseases have been associated with the intestinal microbiota. These include diabetes mellitus (Caricilli *et al*., 2011), stress (Bailey *et al*., 2010), and asthma (van Nimwegen *et al*., 2011). While these diseases also occur in cats, the role of the feline intestinal microbiota has not yet been investigated. It is recognized that differences in microbiota on a phylogenetic and also functional level exist among the various animal species, and more studies are needed to understand the contributions of the microbiota to digestion, immunology and nutrition in every animal species.

Composition of the GI microbiota

Studies in the pre-sequencing era

Traditional culture-based studies have provided fundamental insights into the GI microbial ecology of cats. Several studies have evaluated the bacterial composition of the proximal part of the small intestine, the colon and feces (Osbaldiston and Stowe, 1971; Terada *et al*., 1993; Sparkes *et al*., 1998a, b; Johnston *et al*., 2000, 2001). Tables 1 and 2 summarize the results of those studies. *Bacteroides* spp., *Clostridium* spp., *Enterococcus* spp., *Streptococcus* spp., *Fusobacteria* spp., and *Eubacteria* spp. are the most commonly isolated bacterial groups from the feline GI tract. In general, the microbiota increases in abundance along the GI tract, progressing from stomach to colon. Anaerobic bacterial groups predominate in the distal portions of the GI tract, whereas a more equal distribution of aerobic and anaerobic bacteria is observed in the proximal portions of the GI tract. This distribution of bacterial groups is similar to those observed in humans and other animal species including dogs, a finding that highlights the genetic and environmental factors that play a major role in shaping the host microbiota (Simpson *et al*., 2002; Palmer *et al*., 2007). However, a culture-based study has suggested that the small intestines of cats harbor relatively higher numbers of total bacteria (10^5–10^8 total bacterial colony-forming units (cfu)/ml), with a higher proportion of obligate anaerobic bacteria, when compared with humans and dogs (Johnston *et al*., 2001).

Studies involving molecular methods

Until recently, traditional bacterial culture was the most commonly used method for describing the bacterial groups present in the GI tract of cats. However, because the majority of intestinal bacteria cannot be cultured, a cultivation-based method underestimates total bacterial numbers, and does not allow identification of the majority of bacterial groups present in the GI tract. The recent advances in molecular sequencing technologies have revealed that the mammalian GI tract harbors a highly complex microbial ecosystem, comprising several hundred bacterial genera. Molecular tools allow the identification of previously uncharacterized intestinal microbes and these techniques are also able to provide information about the functionality of the microbiome by means of metagenomics (Swanson *et al*., 2011; Tun *et al*., 2012).

Various methods are available for the characterization of the intestinal microbiota. It is important to understand that all of these methods have strengths and limitations. Ideally, all of these approaches can be used in a complimentary fashion. There is often a discrepancy between the reported abundance of bacterial groups among the various sequencing studies and also when compared with fluorescence *in situ* hybridization (FISH) or metagenomics studies (i.e., *Firmicutes* versus *Bacteroidetes* versus *Actinobacteria*; see below). It is reasonable to attribute some of these differences, as those between FISH- and PCR-based methods, to differences in technology, with the most likely explanation being that the

https://doi.org/10.1017/S1466252312000060 Published online by Cambridge University Press

https://doi.org/10.1017/S1466252312000060 Published online by Cambridge University Press

Table 1. The microbiota present in the feline small intestine based on culture

Osbaldiston and Stowe (1971)		Papasouliotis *et al.* (1998)		Johnston *et al.* (2000)	
Samples: jejunal contents		Samples: duodenal aspirates		Samples: duodenal fluid	
Adult cats (*n*=6)		Adult cats (*n*=25)		Adult cats (*n*=6)	
Organism	Range counts (log counts/g)	Organism	Range counts (log_{10} cfu/ml)	Organism	Range counts (log_{10} cfu/ml)
Total bacteria	N/A	**Total bacteria**	<2.0–8.3	**Total bacteria**	6.3 (mean)
Bacillus	ND – 5.1	**Total aerobes**	<2.0–8.3	**Total aerobes**	5.8 (mean)
Bacteroides	ND	**Total anaerobes**	<2.0–7.5	**Total anaerobes**	5.7 (mean)
Catenabacterium	ND – 4.7	*Bacteroides* spp.	2.3–6.8	*Acinetobacter* spp.	ND – 4.6
Clostridium	ND – 7.0	*Clostridium* spp.	3.8–7.5	*Bacteroides* spp.	4.8–7.6
Enterobacter	ND	Diphtheroids	2.0–6.0	*Clostridium* spp.	3.0–6.0
Enterococcus	4.6–8.2	*E. coli*	2.0–6.0	*Corynebacterium* spp.	ND
Escherichia	ND – 7.4	*Fusobacterium* spp.	2.3–6.6	Diphtheroids	ND – 7.1
Eubacterium	ND – 7.9	*Gram-negative rods*	2.0–7.7	*E. coli*	ND
Lactobacillus	ND – 7.2	*Moraxella* spp.	2.0–5.4	*Eubacterium* spp.	ND – 5.4
Mima	ND – 5.4	*Staphylococcus* spp.	2.0–7.4	*Fusobacterium* spp.	ND – 7.8
Micrococcus	ND – 3.9	*Streptococcus/Enterococcus* spp.	2.0–8.3	*Lactobacillus* spp.	ND – 6.0
Pasteurella	ND – 5.7			*Pasteurella* spp.	3.8–7.3
Proteus	ND – 3.9			*Peptostreptococcus* spp.	ND
Staphylococcus	ND – 5.0			*Propiobacterium* spp.	ND
Streptococcus	ND – 7.5			*Pseudomonas* spp.	ND – 4.4
Veillonella	ND – 6.7			*Staphylococcus* spp.	ND
				Streptococcus spp.	ND
				Unidentified anaerobic bacterium	ND
				Unidentified gram-negative rods	ND – 7.1

Data are cited from Osbaldiston and Stowe (1971), Papasouliotis *et al.* (1998), and Johnston *et al.* (2000). N/A: data were not available, ND: organism was not detected.

Table 2. The microbiota present in the feline large intestine based on culture

Osbaldiston and Stowe (1971)		Terada *et al.* (1993)	
Sample: midcolon contents		Sample: feces	
Adult cats (n=6)		Adult cats (n=8)	
Organism	Range counts (log counts/g)	Organism	Mean counts (log counts/g)
Total bacteria	N/A	Total bacteria	10.7
Bacillus	ND – 9.0	*Bacilli*	4.9
Bacteroides	ND – 5.6	*Bacteroides*	10.4
Catenabacterium	ND – 8.3	*Clostridia*	
Clostridium	ND – 7.7	Lecithinase-positive	9.9
Enterobacter	ND – 7.8	Lecithinase-negative	9.1
Enterococcus	6.7–8.7	*Corynebacteria*	7.5
Escherichia	4.7–8.2	*Enterobacteriaceae*	8.5
Eubacterium	ND – 7.6	*Eubacteria*	9.2
Lactobacillus	0–8.4	*Fusobacteria*	9.1
Pseudomonas	ND	*Lactobacilli*	8.5
Proteus	ND – 4.5	*Peptococcaceae*	9.6
Staphylococcus	ND – 5.1	*Spirochaetaceae*	8.6
Streptococcus	ND – 8.0	*Staphylococci*	5.2
		Streptococci	8.8

Data are cited from Osbaldiston and Stowe (1971) and Terada *et al.* (1993).
N/A: data were not available, ND: organism was not detected.

various methods have different sensitivities and specificities for the examined bacterial groups. For example, sequencing studies in one laboratory may employ different DNA extraction protocols and PCR primers (Baker *et al.*, 2003; Zoetendal *et al.*, 2004). Storage conditions may also cause alterations in sample quality (Ott *et al.*, 2004). Quantitative PCR assays will have bias due to exponential amplification of targets and also because various bacterial phylotypes may have different copy numbers of the 16S rRNA gene, causing preferential amplification of some bacterial groups (Rastogi *et al.*, 2009). However, all molecular methods are generally reproducible within one laboratory, allowing one to draw meaningful conclusions about feline microbiota changes within individual studies. More detailed information about these techniques is provided elsewhere (Suchodolski, 2011). Furthermore, most studies report the analysis of fecal samples, due to their ease of noninvasive collection. Nevertheless, studies have shown that the composition of the GI microbiota varies between anatomical sites (i.e., duodenum versus colon versus feces) and also luminal versus mucosa-adherent tissues (Ott *et al.*, 2004; Suchodolski *et al.*, 2005, 2008; Ritchie *et al.*, 2008). Because of the above-mentioned limitations, caution should be taken when interpreting the reported proportions or abundances of specific bacterial groups across different studies and different methods.

Studies using FISH

FISH allows quantifying bacteria directly by using fluorescent-labeled oligonucleotide probes that target the 16S rRNA. FISH is currently considered to be the most useful method for an accurate quantification of bacterial groups. It can also add information about the morphology and the spatial distribution of the organism (Amann *et al.*, 1995; Moter and Göbel, 2000; Zoetendal *et al.*, 2004; Swidsinski *et al.*, 2005). Unfortunately, this technique is labor intense, and FISH probes need to be designed for the specific bacterial groups of interest. Therefore, FISH is not a high-throughput method and typically is not used for studies involving many samples. However, studies using FISH are providing valuable information about the abundance of total bacteria as well as the abundance of specific bacterial groups in the feline intestine (Table 3). These studies have shown that the total bacterial count is approximately 10.5 $\log_{10}$ cells/gram of feces (Abecia *et al.*, 2010; Jia *et al.*, 2011a). The *Atopobium* group (probe Ato291) including *Coriobacteriaceae*, the *Clostridium* cluster XIVa, and the lactic acid bacteria including *Bifidobacteria* were reported as the most abundant groups in the intestine of kittens and geriatric cats (Abecia *et al.*, 2010; Jia *et al.*, 2011a, b). Similar results for most bacterial groups were also observed in fecal samples of adult cats and cats with IBD (Inness *et al.*, 2007; Abecia *et al.*, 2010). However, *Bifidobacteria* varied to some extent between these latter studies. In the study by Inness *et al.* (2007), *Bifidobacteria* accounted for approximately 11% of total bacteria, while Abecia *et al.* (2010) reported *Bifidobacteria* as accounting for approximately 30% of total counts in healthy adult cats (Inness *et al.*, 2007; Abecia *et al.*, 2010). Of particular interest is that these commonly used probes identify between 40% (Jia *et al.*, 2011a) and 74% (Abecia *et al.*, 2010) of total bacterial counts (i.e., counts obtained with the fluorescent dye DAPI). This suggests that additional

https://doi.org/10.1017/S1466252312000060 Published online by Cambridge University Press

Table 3. Composition of the GI microbiota based on FISH analysis

Probe	Inness *et al.* (2007) Sample: feces Adult cats (*n*=34) Prevalence %	Mean counts log_{10} cells/g	% of total bacteria	Abecia *et al.* (2010) Sample: feces Adult cats (*n*=10) Prevalence %	Mean counts log_{10} cells/g	% of total bacteria
(DAPI)	100	10.28		100	10.06	
Bif164	91.2	9.34	11.48	100	9.54	30.20
Chis150	97.1	7.92	0.44	80	7.22	0.14
Lab158	97.1	8.68	2.51	90	8.21	1.41
Bac303	100	9.07	6.17	N/A	N/A	N/A
SRB687	97.1	7.26	0.10	N/A	N/A	N/A
Erec482	N/A	N/A	N/A	100	8.7	4.37
DSV687	N/A	N/A	N/A	70	6.37	0.02
Ato291	N/A	N/A	N/A	100	9.65	38.90
Clit135	N/A	N/A	N/A	80	7.27	0.16
Rrec584	N/A	N/A	N/A	100	8.21	1.41

Data are cited from Inness *et al.* (2007) and Abecia *et al.* (2010). N/A: data were not available. Oligonucleotide probes (details of each probe are cited from Inness *et al.* 2007; Abecia *et al.*, 2010) – DAPI: all bacteria (nucleic acid stain) Bif164: most *Bifidobacterium* spp., *Parascardovia denticolens;* Chis150: *Clostridium histolyticum* group (comprises organisms belonging to *Clostridium* clusters I and II); Lab158: most *Lactobacillus*, *Leuconostoc*, and *Weissella* spp., *Lactococcus lactis*, all *Vagococcus*, *Enterococcus*, *Melisococcus*, *Tetragenococcus*, *Catellicoccus*, *Pediococcus*, *Paralactobacillus* spp.; Bac303: most *Bacteroides sensu stricto, Prevotella* spp., all *Parabacteroides* spp., *Barnesiella* spp. and *Odoribacter splanchnicus*; SRB687: *Desulfovibrio* spp.; Erec482: most members of *Clostridium* cluster XIVa, *Syntrophococcus sucromutan*, *Bacteroides galacturonicus* and *Bacteroides xylanolyticus*, *Lachnospira pectinschiza, Clostridium saccharolyticum*; DSV687: most *Desulfovibrionales* (excluding *Lawsonia*), many *Desulfuromonales;* Ato291: *Atopobium*, *Collinsella*, *Eggerthella*, *Coriobacterium* and *Cryptobacterium* spp.; Clit135: *Clostridium lituseburense* group (includes *C. difficile*); Rrec584: *Roseburia* spp. and *Eubacterium rectale* (subset of *Clostridium* cluster XIVa).

probes that are able to detect the remaining percentage of bacterial groups will need to be designed, to provide a more complete numerical coverage of the feline intestinal microbiota. Recent sequencing studies have revealed additional groups, and these sequences will aid in the design of FISH probes.

Studies by sequence-based approaches

These methods, targeting specific highly conserved bacterial genes with universal bacterial primers, have been applied to overcome the limitations of culture-based methods for the evaluation of feline microbial ecology. Sequencing methods, either based on the construction of 16S rRNA gene clone libraries or recent high-throughput methods such as 454-pyrosequencing or Illumina sequencing, have allowed the identification of previously uncharacterized bacterial groups. Furthermore, these techniques allow a semi-quantitative assessment of the intestinal microbiota, as the data are expressed as each specific bacterial group as the percentage of all obtained sequences. These sequencing results typically correlate well with confirmatory qPCR analysis. However, there are also potential drawbacks of these methods when using universal bacterial primers. For example, there is evidence that certain bacterial groups (i.e., G+C rich bacteria, Actinobacteria) are underrepresented in 16S rRNA gene sequencing studies (Krogius-Kurikka *et al.*, 2009; Ritchie *et al.*, 2010). This universal primer issue has been discussed extensively, and the use of multiple primer sets has been suggested (Baker *et al.*, 2003; Dethlefsen *et al.*, 2008).

A study employing traditional Sanger sequencing on constructed 16S rRNA gene clone libraries reported five bacterial phyla in the feline GI tract (Ritchie *et al.*, 2008). In this study, *Firmicutes* was the most abundant phylum (68% of clones) in the feces of conventionally raised cats, followed by *Proteobacteria* (14%), *Bacteroidetes* (10%), *Fusobacteria* (5%), and *Actinobacteria* (4%). Within the phylum *Firmicutes, Clostridiales* was the most prevalent bacterial order, representing 40% of clones. *Clostridium* cluster XIVa was the most abundant member of the *Clostridiales* (Ritchie *et al.*, 2008). This study also revealed differences in the composition along the small and large intestine. Another study also demonstrated that a universal primer approach underestimates the prevalence of *Bifidobacterium* spp. in feline fecal samples, and for best characterization of specific bacterial groups of interest it may be useful to employ group-specific primers (Ritchie *et al.*, 2010). Another gene target used for the characterization of the intestinal microbiota is the 60 kDa chaperonin (*cpn*60) gene (Desai *et al.*, 2008). In this study, *Firmicutes* was also the most abundant phylum (41 and 72% of clones from indoor and outdoor cats, respectively), followed by *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria* (Desai *et al.*, 2008).

https://doi.org/10.1017/S1466252312000060 Published online by Cambridge University Press

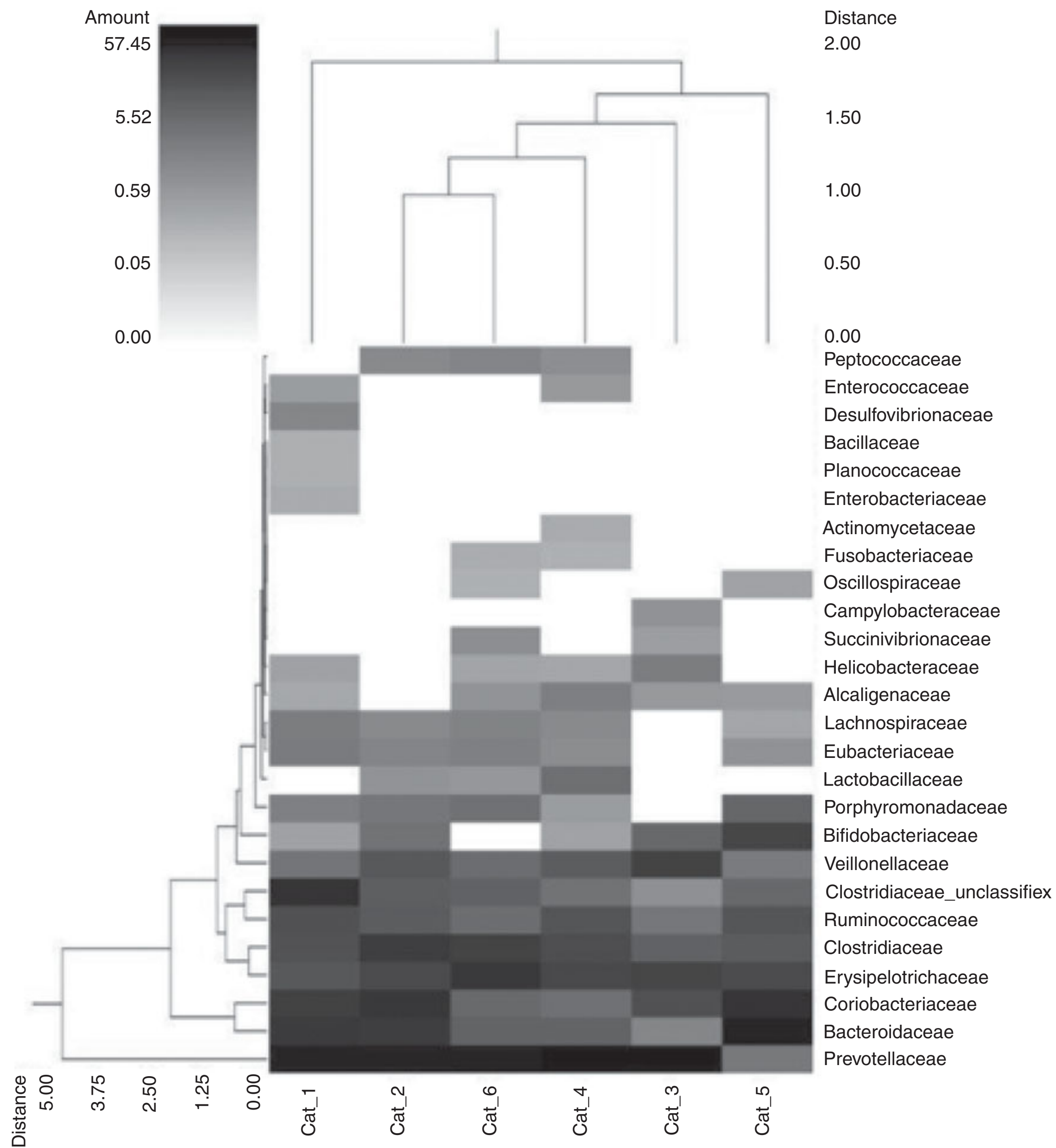

Fig. 1. Dual hierarchal dendogram based on the predominant bacterial families in the GI tract of healthy household cats (454-pyrosequencing of the V4–V6 region of the 16S rRNA gene; unpublished data). The heatmap represents the relative percentage of each family within each sample with legend presented at the top left of the figure. In this study, sequences of the Bacteroidetes and Actinobacteria phyla were the most abundant representatives.

Recently, high-throughput 454-pyrosequencing has been employed for the characterization of the feline GI microbiota. Handl *et al.* (2011) reported *Firmicutes* (92% of sequences) as the most abundant phylum, followed by *Actinobacteria* (7.3%), *Bacteroidetes* (0.45%), and *Fusobacteria* (0.04%). Within the phylum *Firmicutes*, the most abundant bacterial class was *Clostridia* (65%), followed by *Erysipelotrichi* (13%), and *Bacilli* (9%). The class *Clostridia* was dominated by the *Clostridium* clusters XIVa and XI, and *Ruminococcus*. The class *Bacilli* consisted mostly of the order *Lactobacillales*, which was dominated by the genera *Enterococcus* and *Lactobacillus*. The class *Erysipelotrichia* consisted only of the order *Erysipelotrichales*, which mainly comprised the genera *Turicibacter, Catenibacterium*, and *Coprobacillus*. Another study revealed similar distributions of microbial groups in fecal samples of cats (Desai *et al.*, 2008; Garcia-Mazcorro *et al.*, 2011). In contrast, a recent 454-pyrosequencing study performed in our laboratory revealed that the phylum *Bacteroidetes* was the most abundant phylum in fecal samples of five healthy pet cats (unpublished data; Fig. 1). The families *Bacteroidaceae*

https://doi.org/10.1017/S1466252312000060 Published online by Cambridge University Press

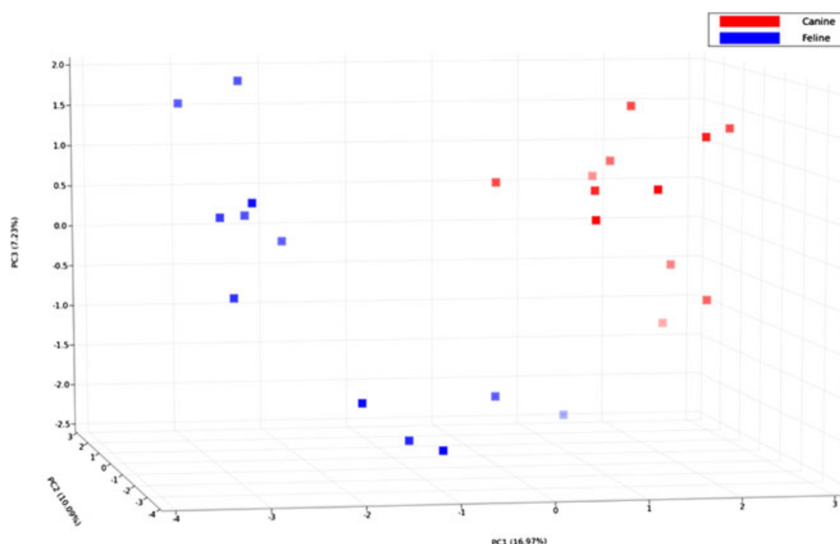

Fig. 2. Principal coordinate analysis plots (PCoA) based on the unweighted Unifrac distance metric illustrating differences in microbial communities present in the GI tract of cats and dogs. Each dot represents the bacterial community of one individual animal. A clear separation was observed by animal species. The data indicate that canine and feline intestinal microbiota differ on a species and/or strain level (PCoA analysis was based on sequences generated by Handl *et al.*, 2011).

and *Prevotellaceae* (phylum *Bacteroidetes*), and *Coriobacteriaceae* (members of the phylum *Actinobacteria*) accounted for approximately 50% of all sequences, followed by *Firmicutes* (29% of sequences) (Fig. 1).

Metagenomics

High-throughput sequencing platforms enable a metagenomics approach (i.e., shotgun sequencing of genomic DNA). This approach yields identification of host and microbial genes present in a sample, and offers an opportunity to assess the functional aspects of the microbiota (Gill *et al.*, 2006). These techniques are very valuable, but due to the current expense of shotgun sequencing, most studies performed yielded only a relatively superficial coverage of the microbiome. Recently, two studies were reported that elucidated the feline intestinal metagenome. One study on pooled fecal samples from five healthy house-hold cats analyzed a total of 152,494 sequences (Tun *et al.*, 2012). The results revealed that the *Bacteroides/Chlorobi* group was the most predominant phylum (68%), followed by *Firmicutes* (13%), *Proteobacteria* (6%), *Actinobacteria* (1.2%), and *Fusobacteria* (0.7%). Within the phylum *Bacteroides/Chlorobi*, the order *Bacteroidetes* was the most abundant, while within the *Firmicutes*, the most abundant bacterial class was *Clostridia* (65%), followed by *Bacilli*, and *Mollicutes*. Another study analyzed a total of 4,192,192 sequences from 12 individual fecal samples from four healthy research colony cats fed three diets (Barry, 2010) and reported the *Bacteroidetes/Chlorobi* group (36.1%) and *Firmicutes* (36.3%) as the predominant phyla, followed by *Proteobacteria* (12.4%) and *Actinobacteria* (7.7%), according to the metagenomics analysis platform MG-RAST.

The metagenomic approach also allows characterizing the microbial genes present. Microbial carbohydrate and protein metabolism accounted for approximately 13 and 9% of the feline metagenome, respectively (Tun *et al.*, 2012). Other major functional metabolic categories included DNA metabolism (8% of the metagenome), virulence factors (7%), and amino acid metabolism (6%) (Tun *et al.*, 2012). Barry (2010) reported that carbohydrates (15%); clustering-based subsystems (14%); protein metabolism (8%); amino acids and derivatives (8%); cell wall and capsule (7%); DNA metabolism (7%); virulence (6%); and cofactors, vitamins, prosthetic groups and pigments (6%), were the major functional metabolic categories present. Clearly, more studies with deeper coverage of the metagenome are warranted in samples from cats with GI disease.

Inter-species and inter-animal differences

Of interest from an ecological perspective and also from interventional aspect is how the intestinal microbiota of cats differs from those of other animal species and also between individual cats. A study involving 106 individuals representing 60 mammalian species showed that microbial communities clustered by host diet and phylogeny, indicating significant differences between different animal species (Ley *et al.*, 2008). In a study using pyrosequencing of the 16S rRNA gene, we compared the microbial communities of dogs and cats (n=12 each) and observed that the feline microbiome appears to be more diverse than the canine microbiome (Handl *et al.*, 2011). Furthermore, bacterial communities of cats and dogs clustered separately (Fig. 2). However,

https://doi.org/10.1017/S1466252312000060 Published online by Cambridge University Press

no particular bacterial groups were significantly associated with either dogs or cats, suggesting that these observed differences between dogs and cats are represented mainly at a bacterial species or strain level.

It is also obvious that the environment influences the intestinal microbiota. Studies using traditional culture methods reported a comparable microbial composition between healthy household cats and research colony cats (Johnston *et al*., 2001). Recent comparative universal gene studies, however, reported differences in microbial composition between conventional cats and one SPF cat (Ritchie *et al*., 2008), and between indoor cats and outdoor cats (Desai *et al*., 2008). Other studies also reported a high inter-individual variation in microbiota composition between cats. In one study, a high percentage of cats harbored the same genera, but only a minor percentage of these cats harbored the same *Bifidobacterium* and *Lactobacillus* species (Ritchie *et al*., 2010). Desai *et al*. also demonstrated that individual cats have a unique abundance of bacterial groups (Desai *et al*., 2008). Figure 1 illustrates the inter-individual differences of the most abundant bacterial families in fecal samples of healthy household cats based on 454-pyrosequencing of the 16S rRNA gene.

Development of GI microbiota

The GI tract of neonates is sterile, but immediately after birth, a life-long process of colonization by foreign microorganisms is initiated (Round and Mazmanian, 2009). Newborn kittens acquire their commensal microbes from the maternal vaginal and fecal and environmental surroundings. Within 24 h after birth, bacterial abundance in fecal samples of kittens has reached levels comparable to those of adults (Buddington and Paulsen, 1998). The general makeup of the intestinal microbiota is relatively similar to that of adults; however, some modifications in the quantitative and the qualitative composition occur over the first few weeks to months of life. A recent study evaluated the development of the fecal microbiota of kittens from 4 weeks to 9 months of age, and observed higher species diversity but also a more variable microbial profile in 4-week-old kittens compared to weaned kittens at 8 weeks of age (Jia *et al*., 2011b). Furthermore, microbiota changes were also observed in relation to diet succession in these growing kittens (Jia *et al*., 2011b). In kittens 8, 12, and 16 weeks of age, Hooda *et al*. (unpublished data) reported that *Firmicutes* was the predominant phylum (70–80% of sequences), followed by moderate *Actinobacteria* and *Fusobacteria* populations, and low *Proteobacteria* and *Bacteroidetes* populations. The microbial community of kittens was greatly impacted by diet in that study. In general, kittens fed a high-protein, low-carbohydrate diet had greater *Fusobacteria* (12.6% versus 0.1%; $P<0.001$) and *Proteobacteria* (3.4% versus 0.5%; $P<0.001$) populations and lower *Actinobacteria* (5.9% versus 24.4%; $P<0.001$) populations than kittens fed a moderate-protein, moderate-carbohydrate diet.

The intestinal microbiome of adult cats is believed to remain relatively stable over time, but is influenced by diet, especially protein and fiber content. While data from human and dog studies suggest that the microbiota of geriatric individuals undergoes some modifications (Benno *et al*., 1992; Woodmansey, 2007), only limited data are available for cats. It is obvious that microbiome changes with age could have an impact on the metabolic activity of the microbes to aid in digestive functions. One study in geriatric cats has revealed that a complex ecosystem is present in older cats, with *Coriobacteriaceae* (phylum *Actinobacteria*), *Clostridium* cluster XIV, *Bifidobacteria* and lactic acid bacteria being the dominant groups in feces (Jia *et al*., 2011a).

Enteropathogens

Diarrhea is one of the most common reasons for cats to be presented to a veterinarian, and infectious causes are typically high on the differential list. Specific enteropathogens, such as *Tritrichomonas foetus*, *Giardia* spp., *Cryptosporidium* spp., enterotoxigenic *Clostridium perfringens*, *Clostridium difficileM, Salmonella* spp., and *Campylobacter jejuni*, have all been associated with GI disease in cats. Several of these potential enteropathogens are commensals in the GI tract and have been isolated at similar frequencies from diarrheic and non-diarrheic animals (e.g., *C. perfringens* and *C. difficile*) (Queen *et al*., 2012). This complicates the clinical interpretation when presumptive enteropathogens are identified based on their presence in feces alone, as the isolation of those organisms from cats does not always indicate the cause for the GI disease. Recently, the American College of Veterinary Internal Medicine (ACVIM) released a consensus statement on the diagnosis, epidemiology, treatment, and control of the primary enteropathogenic bacteria in cats (Marks *et al*., 2011). In this statement, *C. difficile*, *C. perfringens*, *C. jejuni*, *Salmonella* spp., and *Escherichia coli* are listed as being associated with intestinal disease. However, the presence of these organisms does not always correlate with the presence of disease, and care should be taken to not over interpret the isolation of these organisms.

C. difficile is a Gram-positive, spore-forming, anaerobic bacillus that is of critical importance in human medicine, as an increasing number of severe *C. difficile* infections are reported in hospitalized patients (e.g., pseudomembranous colitis) (Bartlett, 2009). The virulence of *C. difficile* is associated with the presence of genes that code for toxins, most notably toxin A (an enterotoxin; *tcd* A) and toxin B (a cytotoxin; *tcd* B). The pathophysiology and role of *C. difficile* in feline enteric disease is much less clear. Isolation rates of *C. difficile* range between

https://doi.org/10.1017/S1466252312000060 Published online by Cambridge University Press

0 and 21% of cats in the general feline population, and 9 and 38% in cats in veterinary hospitals (Marks *et al.*, 2011). The diarrhea incidence often does not differ between cats negative for *C. difficile* and cats that are carriers of this organism (Clooten *et al.*, 2008).

C. perfringens is a Gram-positive, spore-forming, anaerobic bacillus that is a common inhabitant of the intestinal tract, and an important pathogen responsible for a wide spectrum of human and veterinary diseases. The main virulence factors associated with *C. perfringens*-associated diarrhea is an enterotoxin (CPE *Clostridium perfringens enterotoxin*) encoded by the *cpe* gene. CPE induces its toxicity by interaction with intestinal tight junctions, affecting transmembrane pores and leading to alterations in epithelial permeability. *C. perfringens* is a normal commensal of the feline intestine, with reported isolation rates of up to 63% of healthy cats (Queen *et al.*, 2012). Toxigenic *C. perfringens* (i.e., strains that possess the *cpe* gene) can be found by PCR in up to 35% of healthy cats (our unpublished data). However, the enterotoxin CPE has been detected in only 1.9% (Queen *et al.*, 2012) to 6% (our unpublished data) of healthy cats, and in 4.1% (Queen *et al.*, 2012) to 7.2% (our unpublished data) of cats with diarrhea. The overall prevalence of either *C. perfringens* or toxigenic *C. perfringens* strains or the presence of CPE is not significantly different between healthy cats and cats with diarrhea. Therefore, further studies are required to understand the role of this organism and its virulence factor in feline enteric diseases.

Salmonella spp. are Gram-negative, non-spore-forming, motile bacilli. *Salmonella* spp.-related diarrhea is caused by alternating the phosphorylation status of tight junctions resulting in disruption of epithelial barrier function (Viswanathan *et al.*, 2009). *Salmonella* spp. have been isolated from kittens, healthy adult cats, and diseased adult cats at rates up to 51.4, 1.7, and 8.6%, respectively (Van Immerseel *et al.*, 2004). While *Salmonella* spp. are considered pathogens for cats, many infections are subclinical and many healthy cats are shedders. There appear to be a variety of strains that differ in their virulence. Because of the high isolation rate of *Salmonella* in cats, the mere isolation of these organisms from a diarrheic cat does not prove causation (Marks *et al.*, 2011). In cases of uncomplicated diarrhea, only supportive therapy is recommended.

Campylobacter spp. are Gram-negative, curved, motile rods. The genus *Campylobacter* encompasses many species and is considered normal flora of cats. In fact, a recent study using PCR reported that 100% of healthy cats harbored *Campylobacter* spp. (Queen *et al.*, 2012). *Campylobacter helveticus* appears to be the predominant species in the intestine of healthy cats (Suchodolski *et al.*, 2010a, b; Queen *et al.*, 2012). However, the recognized pathogen *C. jejuni* is rarely identified, with a typical prevalence of up to 7% (Queen *et al.*, 2012).

Other bacterial groups that have been associated with GI diseases, but are considered normal flora of cats, include *E. coli* and *Helicobacter* spp. Most strains of *E. coli* are commensals, but enterotoxigenic, enteropathogenic and enterohemorrhagic strains have been associated with intestinal disease (Beutin, 1999). The cat intestine harbors various *Helicobacter* spp., including *Helicobater felis*, *Helicobater heilmannii*, and *Helicobater baculiformis*. In contrast, *Helicobater pylori*, which is associated with gastritis in humans, has not been detected in household cats. Because of the lack of understanding in terms of disease mechanisms and host interactions, the clinical role of those bacteria remains unclear.

Non-specific alterations of GI microbiota

Alterations in the abundance or the composition of intestinal microbiota are considered an important factor in the pathogenesis of GI diseases. Disturbances may result in a dysregulation of adaptive immune responses, and lead to inflammation and/or reduced activity against infection (Round and Mazmanian, 2009). Furthermore, microbial disturbances may result in functional changes of the intestine, leading to altered intestinal permeability, changes in metabolic functions (e.g., deconjugation of bile acids and reduced carbohydrate utilization) that lead to malabsorption and maldigestion. Known consequences of dysbiosis are well recognized in humans, where microbial alterations are associated with GI disorders such as small intestinal bacteria overgrowth (SIBO) or IBD. In humans, SIBO is defined as a heterogeneous syndrome characterized by an increased number and/or abnormal type of bacteria in the small intestine (typically $>10^5$ cfu/ml of small intestinal content) (Bures *et al.*, 2010). The syndrome SIBO *per se* is not recognized in cats, which have high duodenal bacterial counts (10^5–10^8 cfu/ml), and no differences in abundance of bacteria have been observed between healthy and affected cats (Johnston *et al.*, 2001). However, it is well recognized that some cats with chronic diarrhea will respond clinically to antimicrobial treatment, and therefore may be presumptively diagnosed with antibiotic-responsive diarrhea or small intestinal dysbiosis. The latter two terms are currently used synonymously for cats that suffer with a syndrome that resembles SIBO in humans.

There are several consequences of bacterial dysbiosis that may cause intestinal disease (Hall, 2011). For example, bacterial deconjugation of bile acids may lead to malabsorption of fat and lipid-soluble vitamins (Donaldson, 1965; Tabaqchali and Booth, 1967; Shindo *et al.*, 1998). Also, some bacteria produce toxic agents such as ammonia, D-lactate, endotoxin (LPS Lipopolysaccharide), and enterotoxin. Those toxins and bacterial metabolites may cause histological damage to epithelial cells, alterations in membrane permeability, and ultimately lead to inflammation, diarrhea, and malabsorption (McClane, 1996). Also,

https://doi.org/10.1017/S1466252312000060 Published online by Cambridge University Press

competition for nutrients and vitamins between host and bacteria, and also between beneficial and harmful bacteria can reduce substrates available for the host (Welkos *et al*., 1981). Depletions in vitamin B_{12} and also increases in D-lactate are potential consequences of intestinal dysbiosis in cats (Packer *et al*., 2012).

Chronic enteropathies

IBD is defined as an inflammation of the GI tract with persistent or recurrent GI signs (Simpson and Jergens, 2011). A combination of altered intestinal microbial ecosystem, an underlying genetic susceptibility of the host, and dietary and/or environmental factors are suspected to be the main contributing factors in the pathogenesis of IBD. Recent advances in microbiome research have allowed a deeper understanding of compositional changes in IBD, although only a few studies are available in cats, and most data have been reported for humans or dogs (Frank *et al*., 2007; Packey and Sartor, 2009). Studies using sequencing techniques have revealed that microbiota changes in human IBD typically involve a lower abundance of *Firmicutes* and *Bacteroides*, and a higher abundance of *Proteobacteria* compared to healthy individuals (Seksik, 2010). Similar changes have also been observed in canine IBD (Xenoulis *et al*., 2008; Suchodolski *et al*., 2010a, b). While sequencing methods have not been reported for the characterization of feline IBD, a study using FISH has revealed an increase in *Enterobacteriaceae* in duodenal biopsies of cats with IBD (Janeczko *et al*., 2008). Furthermore, a relationship between increased bacterial numbers and the severity of histological inflammation was observed. Several studies compared the fecal microbiota between cats with IBD and healthy control cats. In one study, cats with IBD had lower FISH counts for total bacteria, *Bacteroides* spp., and *Bifidobacterium* spp., but higher counts of *Desulfovibrio* spp. compared to healthy cats (Inness *et al*., 2007). *Desulfovibrio* spp. are a sulphate-reducing bacterial group and are able to produce hydrogen sulphide, which may be associated with the pathogenesis of feline IBD. However, another study did not identify significant differences in FISH counts between cats with IBD and controls, although the same bacterial groups were targeted (Abecia *et al*., 2010).

Functional aspects of the feline GI microbiome

The metabolic functions of micro-organisms are assumed to be one of the main evolutionary driving forces behind the coevolution of GI microbiota with their host (Van den Abbeele *et al*., 2011). Various studies have shown that specific bacterial populations provide nutritional benefits to the host. The primary end products of bacterial fermentation of non-digestible dietary fibers, such as short-chain fatty acids (SCFA), have been the center of attention due to their anti-inflammatory effects and their importance as an energy source for intestinal epithelial cells. Studies in humans have revealed associations between fecal SCFA concentrations and GI disorders such as IBD and colorectal cancer (O'Keefe *et al*., 2009), and SCFA are thought to confer a protective role against further disease progress (Hamer *et al*., 2008). Similarly, studies in cats have revealed the importance of SCFA for proper intestinal function, demonstrating their impact on colonic motility and energy source (Brosey *et al*., 2000; Rondeau *et al*., 2003).

Several nutritional studies in research cats have evaluated the impact of feeding of various fiber sources or prebiotics on fecal SCFA concentrations and bacterial populations (Sunvold *et al*., 1994; Barry *et al*., 2010; Kanakupt *et al*., 2011). Short-chain fructo-oligosaccharides (scFOS) and galactooligosaccharides (GOS), both established prebiotics in humans, have recently been tested for their prebiotic potential in cats. Using qPCR, Barry *et al*. (2010) reported increased bifidobacteria and decreased *E. coli* populations in adult cats fed 4% scFOS compared to those fed control diets or 4% pectin. In cats fed scFOS, fecal butyrate was also increased compared to those fed the control diet. In that study, cats fed 4% pectin had greater fecal *C. perfringens*, *E. coli*, and lactobacilli populations and fecal acetate, propionate, butyrate, and total SCFA concentrations compared to cats fed the control diet. Kanakupt *et al*. (2011) reported similar results in cats fed 0.5% scFOS, 0.5% GOS, or 0.5% scFOS+0.5% GOS, with all three treatments increasing fecal bifidobacteria populations compared to controls, as assessed by qPCR. Cats fed scFOS+GOS also had increased fecal acetate, butyrate, and total SCFA concentrations.

While the results above are promising, similar studies using high-throughput techniques are needed to assess changes to the entire microbiome on a phylogenetic and functional level. More research is also required to identify other potential prebiotics, determine effective prebiotic dose, and test their efficacy in clinical populations. Very limited information is available about associations between intestinal SCFA concentrations and feline GI disorders. In contrast to studies in humans and dogs, there is still limited information available on the extent of intestinal dysbiosis in feline GI disease. This area deserves future attention, as it is likely that an intestinal dysbiosis, characterized by similar changes in intestinal bacterial groups as observed in human or canine GI disease (i.e., reductions in *Clostridium* clusters IV and XIVa), would have an impact on SCFA concentrations (Hansen *et al*., 2010).

Fungi, protozoa, archaea, viruses

Bacteria are by far the most abundant constituents of the mammalian GI tract. However, it is now recognized

https://doi.org/10.1017/S1466252312000060 Published online by Cambridge University Press

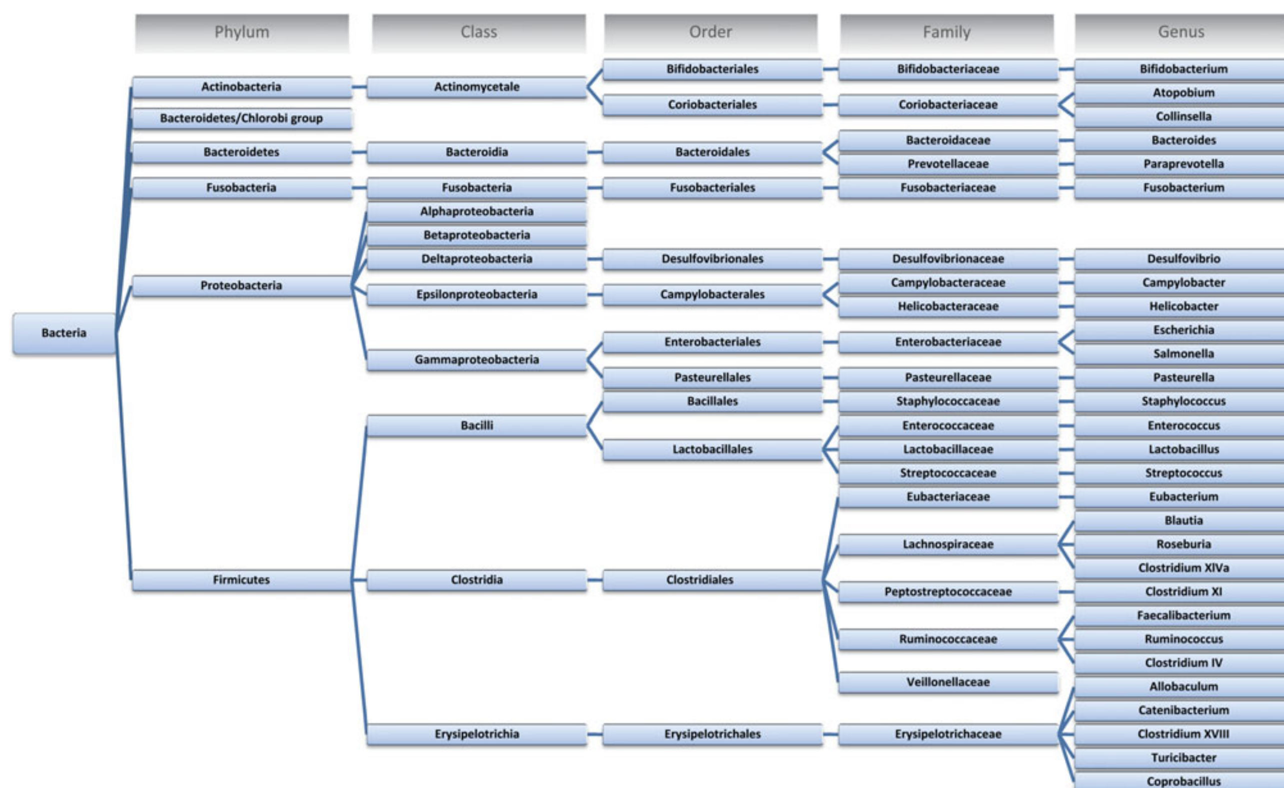

Fig. 3. Taxonomic lineages summarizing the predominant bacterial groups identified in the feline GI tract using molecular methods (Inness *et al.*, 2007; Desai *et al.*, 2008; Ritchie *et al.*, 2008; Abecia *et al.*, 2010; Handl *et al.*, 2011; Tun *et al.*, 2012).

that the gut harbors a highly diverse population of fungal organisms, protozoa, archaea, viruses, and bacteriophages. FISH and shotgun sequencing studies of human and canine fecal DNA have estimated the abundance of fungal organisms and archaea as <2% of total microbiota (Scanlan and Marchesi, 2008; Swanson *et al.*, 2011). A recent metagenomic approach estimated that the feline GI microbiota constitutes 0.02% fungi, 0.09% archaea, and 0.09% viruses (Tun *et al.*, 2012). The detected viruses were 99% bacteriophages (Tun *et al.*, 2012). Data from another feline metagenomics project were quite similar, with fungi, archaea, and viruses constituting approximately 0.3, 1, and 0.25% of the sequences, respectively (Barry, 2010). Using archaea-specific primers, we recently evaluated fecal samples of 10 healthy cats (Suchodolski, 2011; Suchodolski *et al.*, 2011). The most commonly observed archaeal phyla were *Crenarchaeota* and *Euryarchaeota*, and the most abundant families were *Desulfurococcaceae* (54.8% of all sequences), *Methanobacteriaceae* (40.6%), *Methanosarcinaceae* (5.0%), and *Halobacteriaceae* (2.7%). Fungi were described using pyrosequencing of the fungal 18S rRNA gene in pooled fecal samples of cats (Handl *et al.*, 2011), with *Aspergillus* and *Saccharomyces* being the most abundant fungal genera. Clearly, more work is required in characterizing inter-individual differences in fungal and archaeal phylotypes, and how these are affected by dietary influences and their role in GI health and disease.

Future directions

As mentioned above, quantitative and qualitative alterations of intestinal microbiota are deeply associated with the etiopathogenesis of feline intestinal diseases. It is clear that a balanced GI microbiota ecosystem is crucial to promote feline GI health. However, it still remains to be determined whether the observed disturbances of GI microbiota are a cause or the result of the disease process. To answer this complex question, a better understanding of host–bacterial interactions and functional aspects of the microbiota is necessary.

We have made great progress in understanding ecological principles of the intestinal microbiota in various animal species. However, our understanding of the feline microbiota is still rudimentary. The continuing affordability of high-throughput sequencing technology (e.g., 454- pyrosequencing, Illumina, and Ion Torrent) will allow us to gain a better insight into how the microbiota is influenced by dietary and environmental factors on a phylogenetic and metagenomic level. However, to better understand the host–microbes interactions, we will also need to explore changes in microbial metabolic functions, and also host responses through transcriptomics. This will require multi-center studies with experts in the field of veterinary gastroenterology, nutrition, molecular sciences, and bioinformatics. Furthermore, we need to more accurately quantify the

https://doi.org/10.1017/S1466252312000060 Published online by Cambridge University Press

bacterial groups in the feline GI tract. The results of pyrosequencing have yielded a good estimate of the various bacterial groups present in the GI tract (Fig. 3). These sequence data are now available and should allow for the design of FISH probes that would be a useful tool to more accurately quantify the various bacterial populations. Further studies are also needed to explore the mucosa-adherent microbiota in various GI disorders in more detail. And finally, we should attempt to explore the intestinal microbiome in cats with various extra-alimentary diseases that have been associated with alterations of intestinal microbiota, such as diabetes mellitus (Caricilli *et al*., 2011), stress (Bailey *et al*., 2010), and asthma (van Nimwegen *et al*., 2011). All of this information will be useful to design dietary or environmental strategies that may alter the intestinal microbiota, on a phylogenetic or functional level, resulting in a beneficial outcome for the host.

References

Abecia LH, Hoyles L, Khoo C, Frantz N and McCartney AL (2010). Effects of a novel galactooligosaccharide on the faecal microbiota of healthy and inflammatory bowel disease cats during a randomized, double-blind, cross-over feeding study. *International Journal of Probiotics and Prebiotics* **5**: 61–68.

Amann R, Ludwig W and Schleifer K (1995). Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiological Reviews* **59**: 143–169.

Bailey MT, Dowd SE, Parry NM, Galley JD, Schauer DB and Lyte M (2010). Stressor exposure disrupts commensal microbial populations in the intestines and leads to increased colonization by *Citrobacter rodentium*. *Infection and Immunity* **78**: 1509–1519.

Baker G, Smith J and Cowan D (2003). Review and re-analysis of domain-specific 16S primers. *Journal of Microbiological Methods* **55**: 541–555.

Barry KA (2010). *Indices of gut health and intestinal microbial ecology of the cat as affected by ingestion of select carbohydrates varying in fermentative capacity*. PhD Thesis, University of Illinois.

Barry KA, Wojcicki BJ, Middelbos IS, Vester BM, Swanson KS and Fahey Jr GC (2010). Dietary cellulose, fructooligosaccharides, and pectin modify fecal protein catabolites and microbial populations in adult cats. *Journal of Animal Science* **88**: 2978–2987.

Bartlett JG (2009). *Clostridium difficile* infection: historic review. *Anaerobe* **15**: 227–229.

Benno Y, Nakao H, Uchida K and Mitsuoka T (1992). Impact of the advances in age on the gastrointestinal microflora of beagle dogs. *Journal of Veterinary Medical Science* **54**: 703–706.

Beutin, L (1999). *Escherichia coli* as a pathogen in dogs and cats. *Veterinary Research* **30**: 285–298.

Brosey BP, Hill RC and Scott KC (2000). Gastrointestinal volatile fatty acid concentrations and pH in cats. *American Journal of Veterinary Research* **61**: 359–361.

Buddington RK and Paulsen DB (1998). Development of canine and feline gastrointestinal tract. *In*: Carey DP and Reinhart GA (eds.) *1998 IAMS Nutrition Symposium*. Orange Frazer Press. Wilmington, Ohio, USA.

Bures J, Cyrany J, Kohoutova D, Förstl M, Rejchrt S, Kvetina J, Vorisek V and Kopacova M (2010). Small intestinal bacterial overgrowth syndrome. *World Journal of Gastroenterology* **16**: 2978–2990.

Bybee SN, Scorza AV and Lappin MR (2011). Effect of the probiotic *Enterococcus faecium* SF68 on presence of diarrhea in cats and dogs housed in an animal shelter. *Journal of Veterinary Internal Medicine* **25**: 856–860.

Caricilli AM, Picardi PK, de Abreu LL, Ueno M, Prada PO, Ropelle ER, Hirabara SM, Castoldi Â, Vieira P, Camara NO, Curi R, Carvalheira JB and Saad MJ (2011). Gut microbiota is a key modulator of insulin resistance in TLR 2 knockout mice. *PLoS Biology* **9**, e1001212.

Clooten J, Kruth S, Arroyo L and Weese JS (2008). Prevalence and risk factors for *Clostridium difficile* colonization in dogs and cats hospitalized in an intensive care unit. *Veterinary Microbiology* **129**: 209–214.

Desai AR, Musil KM, Carr AP and Hill JE (2008). Characterization and quantification of feline fecal microbiota using *cpn*60 sequence-based methods and investigation of animal-to-animal variation in microbial population structure. *Veterinary Microbiology* **137**: 120–128.

Dethlefsen L, Huse S, Sogin ML and Relman DA (2008). The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biology* **6**, e280.

Donaldson RM (1965). Studies on the pathogenesis of steatorrhea in the blind loop syndrome. *Journal of Clinical Investigation* **44**: 1815–1825.

Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE and Relman DA (2005). Diversity of the human intestinal microbial flora. *Science* **308**: 1635–1638.

Frank DN, Amand ALS, Feldman RA, Boedeker EC, Harpaz N and Pace NR (2007). Molecular–phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 13780–13785.

Garcia-Mazcorro JF, Lanerie DJ, Dowd SE, Paddock CG, Grützner N, Steiner JM, Ivanek R and Suchodolski JS (2011). Effect of a multi-species synbiotic formulation on fecal bacterial microbiota of healthy cats and dogs as evaluated by pyrosequencing. *FEMS Microbiology Ecology* **78**: 542–554.

Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM and Nelson KE (2006). Metagenomic analysis of the human distal gut microbiome. *Science* **312**: 1355–1359.

Gronvold AMR, L'Abee-Lund TM, Sorum H, Skancke E, Yannarell AC and Mackie RI (2010). Changes in fecal microbiota of healthy dogs administered amoxicillin. *FEMS Microbiology Ecology* **71**: 313–326.

Hall EJ (2011). Antibiotic-Responsive Diarrhea in Small Animals. *Veterinary Clinics of North America: Small Animal Practice* **41**: 273–286.

Hamer HM, Jonkers D, Venema K, Vanhoutvin S, Troost FJ and Brummer RJ (2008). Review article: the role of butyrate on colonic function. *Alimentary Pharmacology and Therapeutics* **27**: 104–119.

Handl S, Dowd SE, Garcia-Mazcorro JF, Steiner JM and Suchodolski JS (2011). Massive parallel 16S rRNA gene pyrosequencing reveals highly diverse fecal bacterial and fungal communities in healthy dogs and cats. *FEMS Microbiology Ecology* **76**: 301–310.

Hansen J, Gulati A and Sartor RB (2010). The role of mucosal immunity and host genetics in defining intestinal commen-

https://doi.org/10.1017/S1466252312000060 Published online by Cambridge University Press

sal bacteria. *Current Opinion in Gastroenterology* **26**: 564–571.

Hart ML, Suchodolski JS, Steiner JM and Webb CG (2012). Open-label trial of a multi-strain synbiotic in cats with chronic diarrhea. *Journal of Feline Medicine and Surgery* doi: 10.1177/1098612X11434386. **14**(4): 240–245.

Inness VL, McCartney AL, Khoo C, Gross KL and Gibson GR (2007). Molecular characterisation of the gut microflora of healthy and inflammatory bowel disease cats using fluorescence *in situ* hybridisation with special reference to *Desulfovibrio* spp. *Journal of Animal Physiology and Animal Nutrition* **91**: 48–53.

Janeczko S, Atwater D, Bogel E, Greiter-Wilke A, Gerold A, Baumgart M, Bender H, McDonough PL, McDonough SP, Goldstein RE and Simpson KW (2008). The relationship of mucosal bacteria to duodenal histopathology, cytokine mRNA, and clinical disease activity in cats with inflammatory bowel disease. *Veterinary Microbiology* **128**: 178–193.

Jia J, Frantz N, Khoo C, Gibson GR, Rastall RA and McCartney AL (2011a). Investigation of the faecal microbiota of geriatric cats. *Letters in Applied Microbiology* **53**: 288–293.

Jia J, Frantz N, Khoo C, Gibson GR, Rastall RA and McCartney AL (2011b). Investigation of the faecal microbiota of kittens: monitoring bacterial succession and effect of diet. *FEMS Microbiology Ecology* **78**: 395–404.

Johnston KL, Lamport AI, BallŠvre OP and Batt RM (2000). Effects of oral administration of metronidazole on small intestinal bacteria and nutrients of cats. *American Journal of Veterinary Research* **61**: 1106–1112.

Johnston KL, Swift NC, Forster-van Hijfte M, Rutgers HC, Lamport A, Ballevre O and Batt RM (2001). Comparison of the bacterial flora of the duodenum in healthy cats and cats with signs of gastrointestinal tract disease. *Journal of the American Veterinary Medical Association* **218**: 48–51.

Kanakupt K, Vester Boler BM, Dunsford BR and Fahey Jr GC (2011). Effects of short-chain fructooligosaccharides and galactooligosaccharides, individually and in combination, on nutrient digestibility, fecal fermentative metabolite concentrations, and large bowel microbial ecology of healthy adults cats. *Journal of Animal Science* **89**: 1376–1384.

Krogius-Kurikka L, Kassinen A, Paulin L, Corander J, Mäkivuokko H, Tuimala J and Palva A (2009). Sequence analysis of percent G+C fraction libraries of human faecal bacterial DNA reveals a high number of Actinobacteria. *BMC Microbiology* **9**: 68.

Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML, Tucker TA, Schrenzel MD, Knight R and Gordon JI (2008). Evolution of mammals and their gut microbes. *Science* **320**: 1647–1651.

Marks SL, Rankin SC, Byrne BA and Weese JS (2011). Enteropathogenic bacteria in dogs and cats: diagnosis, epidemiology, treatment, and control. *Journal of Veterinary Internal Medicine* **25**: 1195–1208.

McClane BA (1996). An overview of *Clostridium perfringens* enterotoxin. *Toxicon* **34**: 1335–1143.

Moter A and Göbel UB (2000). Fluorescence *in situ* hybridization (FISH) for direct visualization of microorganisms. *Journal of Microbiological Methods* **41**: 85–112.

O'Keefe SJ, Ou J, Aufreiter S, O'Connor D, Sharma S, Sepulveda J, Fukuwatari T, Shibata K and Mawhinney T (2009). Products of the colonic microbiota mediate the effects of diet on colon cancer risk. *Journal of Nutrition* **139**: 2044–2048. Orange Frazer Press. Wilmington, Ohio, USA

Osbaldiston GW and Stowe EC (1971). Microflora of alimentary tract of cats. *American Journal of Veterinary Research* **32**: 1399–1405.

Ott SJ, Musfeldt M, Timmis KN, Hampe J, Wenderoth DF and Schreiber S (2004). In vitro alterations of intestinal bacterial microbiota in fecal samples during storage. *Diagnostic Microbiology and Infectious Disease* **50**: 237–245.

Packer RA, Moore GE, Chang CY, Zello GA, Abeysekara S, Naylor JM, Steiner JM, Suchodolski JS and O'Brien DP (2012). Serum D-lactate concentrations in cats with gastrointestinal disease. *Journal of Veterinary Internal Medicine*, doi: 10.1111/j.1939-1676.2012.00936.x. [Epub ahead of print]. **22**(3): 292–301.

Packey CD and Sartor RB (2009). Commensal bacteria, traditional and opportunistic pathogens, dysbiosis and bacterial killing in inflammatory bowel diseases. *Current Opinion in Infectious Diseases* **22**: 292–301.

Palmer C, Bik EM, DiGiulio DB, Relman DA and Brown PO (2007). Development of the human infant intestinal microbiota. *PLoS Biology* **5**, e177.

Queen EV, Marks SL and Farver TB (2012). Prevalence of selected bacterial and parasitic agents in feces from diarrheic and healthy control cats from Northern California. *Journal of Veterinary Internal Medicine* **26**: 54–60.

Rastogi R, Wu M, Dasgupta I and Fox GE (2009). Visualization of ribosomal RNA operon copy number distribution. *BMC Microbiology* **9**, 208.

Ritchie LE, Burke KF, Garcia-Mazcorro JF, Steiner JM and Suchodolski JS (2010). Characterization of fecal microbiota in cats using universal 16S rRNA gene and group-specific primers for *Lactobacillus* and *Bifidobacterium* spp. *Veterinary Microbiology* **144**: 140–146.

Ritchie LE, Steiner JM and Suchodolski JS (2008). Assessment of microbial diversity along the feline intestinal tract using 16S rRNA gene analysis. *FEMS Microbiology Ecology* **66**: 590–598.

Rondeau MP, Meltzer K, Michel KE, McManus CM and Washabau RJ (2003). Short chain fatty acids stimulate feline colonic smooth muscle contraction. *Journal of Feline Medicine and Surgery* **5**: 167–173.

Round JL and Mazmanian SK (2009). The gut microbiota shapes intestinal immune responses during health and disease. *Nature Reviews Immunology* **9**: 313–323.

Scanlan PD and Marchesi JR (2008). Micro-eukaryotic diversity of the human distal gut microbiota: qualitative assessment using culture-dependent and -independent analysis of faeces. *ISME Journal* **2**: 1183–1193.

Seksik P (2010). Gur mircrobiota and IBD. Gastroenterologie clinique et biologique **34**: S44–S51.

Shindo K, Machida M, Koide K, Fukumura M and Yamazaki R (1998). Deconjugation ability of bacteria isolated from the jejunal fluid of patients with progressive systemic sclerosis and its gastric pH. *Hepato-gastroenterology* **45**.

Simpson J, Martineau B, Jones W, Ballam J and Mackie R (2002). Characterization of fecal bacterial populations in canines: effects of age, breed and dietary fiber. *Microbial Ecology* **44**: 1–12.

Simpson KW and Jergens AE (2011). Pitfalls and progress in the diagnosis and management of canine inflammatory bowel disease. *Veterinary Clinics of North America: Small Animal Practice* **41**: 381–398.

Sparkes AH, Papasouliotis K, Sunvold G, Werrett G, Clarke C, Jones M, Gruffydd-Jones TJ and Reinhart G (1998a). Bacterial flora in the duodenum of healthy cats, and effect of dietary supplementation with fructo-oligosaccharides. *American Journal of Veterinary Research* **59**: 431–435.

Sparkes AH, Papasouliotis K, Sunvold G, Werrett G, Gruffydd-Jones EA, Egan K, Gruffydd-Jones TJ and Reinhart G (1998b). Effect of dietary supplementation with fructo-oligosaccharides on fecal flora of healthy cats. *American Journal of Veterinary Research* **59**: 436–440.

https://doi.org/10.1017/S1466252312000060 Published online by Cambridge University Press

Suchodolski JS, Ruaux CG, Steiner JM, Fetz K and Williams DA (2005). Assessment of the qualitative variation in bacterial microflora among compartments of the intestinal tract of dogs by use of a molecular fingerprinting technique. *American Journal of Veterinary Research* **66**: 1556–1562.

Suchodolski JS, Camacho J and Steiner JM (2008). Analysis of bacterial diversity in the canine duodenum, jejunum, ileum, and colon by comparative 16S rRNA gene analysis. *FEMS Microbiology Ecology* **66**: 567–578.

Suchodolski JS, Dowd SE, Westermarck E, Steiner JM, Wolcott RD, Spillmann T and Harmoinen JA (2009). The effect of the macrolide antibiotic tylosin on microbial diversity in the canine small intestine as demonstrated by massive parallel 16S rRNA gene sequencing. *BMC Microbiology* **9**, 210.

Suchodolski JS, Xenoulis PG, Paddock CG, Steiner JM and Jergens AE (2010a). Molecular analysis of the bacterial microbiota in duodenal biopsies from dogs with idiopathic inflammatory bowel disease. *Veterinary Microbiology* **142**: 394–400.

Suchodolski JS, Gossett NM, Aicher KM, Heilmann RM, Xenoulis PG and Steiner JM (2010b). Molecular assay for the detection of *Campylobacter* spp. in canine and feline fecal samples. *Journal of Veterinary Internal Medicine* **24**: 748–749.

Suchodolski JS (2011). Intestinal Microbiota of Dogs and Cats: a Bigger World than We Thought. *Veterinary Clinics of North America: Small Animal Practice* **41**: 261–272.

Suchodolski JS, Steinberg BR, Butterfield A and Steiner JM (2011). Prevalence of archaea in the gastrointestinal tract of dogs and cats. *21th ECVIM-CA Congress*, 2011, p. 236.

Sunvold GD, Titgemeyer EC, Bourquin LD, Fahey Jr GC and Reinhart GA (1994). Fermentability of selected fibrous substrates by cat fecal microflora. *Journal of Nutrition* **124**: 2721S–2722S.

Swanson KS, Dowd SE, Suchodolski JS, Middelbos IS, Vester BM, Barry KA and Fahey Jr GC (2011). Phylogenetic and gene-centric metagenomics of the canine intestinal microbiome reveals similarities with humans and mice. *ISME Journal* **5**: 639–649.

Swidsinski A, Weber J, Loening-Baucke V, Hale LP and Lochs H (2005). Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. *Journal of Clinical Microbiology* **43**: 3380–3389.

Tabaqchali S and Booth CC (1967). Relationship of the intestinal bacterial flora to absorption. *British Medical Bulletin* **23**: 285–290.

Terada A, Hara H, Kato S, Kimura T, Fujimori I, Hara K, Maruyama T and Mitsuoka T (1993). Effect of lactosucrose (4G-beta-D-galactosylsucrose) on fecal flora and fecal putrefactive products of cats. *Journal of Veterinary Medical Science* **55**: 291–295.

Tun HM, Brar MS, Khin N, Jun L, Hui RK, Dowd SE and Leung FC (2012). Gene-centric metagenomics analysis of feline intestinal microbiome using 454 junior pyrosequencing. *Journal of Microbiological Methods* **88**: 369–376.

Van den Abbeele P, Van de Wiele T, Verstraete W and Possemiers S (2011). The host selects mucosal and luminal associations of coevolved gut microorganisms: a novel concept. *FEMS Microbiology Reviews* **35**: 681–704.

Van Immerseel F, Pasmans F, De Buck J, Rychlik I, Hradecka H, Collard JM, Wildemauwe C, Heyndrickx M, Ducatelle R and Haesebrouck F (2004). Cats as a risk for transmission of antimicrobial drug-resistant *Salmonella*. *Emerging Infectious Diseases* **10**: 2169–2174.

Van Nimwegen FA, Penders J, Stobberingh EE, Postma DS, Koppelman GH, Kerkhof M, Reijmerink NE, Dompeling E, van den Brandt PA, Ferreira I, Mommers M and Thijs C (2011). Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy. *Journal of Allergy and Clinical Immunology* **128**: 948–955, e1–e3.

Vester BM, Dalsing BL, Middelbos IS, Apanavicius CJ, Lubbs DC and Swanson KS (2009). Faecal microbial populations of growing kittens fed high- or moderate-protein diets. *Archives of Animal Nutrition* **63**: 254–265.

Viswanathan VK, Hodges K and Hecht G (2009). Enteric infection meets intestinal function: how bacterial pathogens cause diarrhoea. *Nature Reviews Microbiology* **7**: 110–119.

Welkos SL, Toskes PP and Baer H (1981). Importance of anaerobic bacteria in the cobalamin malabsorption of the experimental rat blind loop syndrome. *Gastroenterology* **80**: 313–320.

Woodmansey EJ (2007). Intestinal bacteria and ageing. *Journal of Applied Microbiology* **102**: 1178–1186.

Xenoulis PG, Palculict B, Allenspach K, Steiner JM, Van House AM and Suchodolski JS (2008). Molecular–phylogenetic characterization of microbial communities imbalances in the small intestine of dogs with inflammatory bowel disease. *FEMS Microbiology Ecology* **66**: 579–589.

Zoetendal EG, Collier CT, Koike S, Mackie RI and Gaskins HR (2004). Molecular ecological analysis of the gastrointestinal microbiota: a review. *Journal of Nutrition* **134**: 465–472.

https://doi.org/10.1017/S1466252312000060 Published online by Cambridge University Press