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The canine gastrointestinal microbiota: early studies and research frontiers

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ABSTRACT

The canine gut microbiota is a complex microbial population that is potentially related to metabolism, immunologic activity and gastrointestinal (GI) diseases. Early studies revealed that the canine gut microbiota was dynamic, and bacterial populations in the adjacent gut segments were similar, with anaerobes predominating. Metagenomics analysis revealed that nutrient contents in the diet modulated bacterial populations and metabolites in the canine gut. Further research revealed significant correlations between dietary factors and canine gut core microbiomes. Canine GI diseases are closely correlated with gut microbiota dysbiosis and metabolic disorders. Probiotic-related therapies can effectively treat canine GI diseases. Recent studies have revealed that the canine gut microbiota is similar to the human gut microbiota, and dietary factors affect both. Studying canine intestinal microorganisms enables clarifying changes in the canine intestinal bacteria under different conditions, simulating human diseases in dog models, and conducting in-depth studies of the interactions between intestinal bacteria and disease.

Introduction

From basic research to clinical applications, the importance of the gut microbiota has rapidly evolved in recent years.[1] With the applications of metagenomics, metabolomics and culturomics in investigating the gut microbiota, many researchers have reported the important roles of the gut microbiota in contributing to metabolic functions, immunologic activity and neurodevelopment.[2,3] The gut microbiota has been widely studied in humans and laboratory rodents. Canines are common laboratory animals that are widely used in different fields. To date, limited reviews have been published on the canine gut microbiota. Here, we describe findings on the canine gut microbiota from early studies as well as the effects of different foods, models of gastrointestinal diseases and probiotics on the canine gut microbiota.

Early studies on the canine gut microbiota

Canines, especially beagles, are widely used in scientific research. In as early as 1977, the gut microbiomes in various regions of the gastrointestinal (GI) tracts of beagles were assessed by culturing strict anaerobes and making observations via optical and scanning electron microscopy. All canines possess complicated microbiotas in their colons, with anaerobes predominating. One study showed that the canine microbiota became altered and increased in complexity when dogs were housed in a locked environment. These alterations were closely associated with the gastrointestinal epithelial cells and crypts of Lieberkuhn.[4] Authors have also predicted that less predominant microbial species in the GI tract can change over time when animals are housed in locked environments. Anaerobic bacteria are identified by Gram staining, biochemical tests, and volatile and nonvolatile fatty acids via gas chromatography. Bacterial isolates were difficult to identify in large quantities until PCR techniques emerged.

Denaturing gradient gel electrophoresis (DGGE) was first used in 1993. DGGE is used to analyze the genetic diversity of complex microbial populations such as environmental or fecal samples.[5] The gut microbiota comprises complex microbial populations that can be analyzed via DGGE.[6] Molecular fingerprinting via PCR-DGGE

has revealed different diversity levels in fecal samples from different animals. Here, we present research findings on the canine gut microbiota, which were obtained using DGGE.

One study used DGGE to evaluate the differences in bacterial compositions of the intestinal compartments of canines housed in similar environments and fed identical diets. These authors found significantly more similarities in microbial diversity levels between neighboring intestinal regions than between non-neighboring regions.[7] Another study using canine duodenal fluid indicated that the canine small intestine had a highly diverse microbiota with marked differences in diversity between individual dogs. The results were duplicated and highly reproducible. Each dog's duodenum, jejunum, ileum, colon and rectum had significantly different gut microbiotas, and the colon and rectum had higher bacterial diversity than did the other gut segments.[8] Although the gut segments significantly differed in their bacterial populations, the adjacent gut segments were more similar than were the separated gut segments.

Percent G + C profiling is another approach to analyzing bacterial community profiles.[9] One study used percent G + C profiling to determine the relationship between the beagle gut microbiota and the contents of animal-derived protein and carbohydrates from feed. Beagles were divided into the high-carbohydrate (HC), high-protein (HP) and dry commercial (DC; control) groups. The HC and HP groups were fed formulated HC (starch: 438 g/kg) and HP (crude protein: 609 g/kg) diets for one week. Compared with the DC group, the HC and HP groups had decreased microbial diversity with different representative bacteria at the order level. Diversity among Fusobacteriales was significantly increased in the HP group, and the dogs in this group had diarrhea. In the HC group, the most abundant sequences belonged to the order Clostridiales.[10] The altered bacterial diversity may have played a role in inducing diarrhea, thus revealing the potential relationships between the gut microbiota and gastrointestinal diseases. A modified strategy combines percent G + C profiling (GC fractionation) with DGGE (GC-DGGE). GC-DGGE enhances microbiota diversity assessments and detection of minority microbiome communities in fecal samples.[11] Using molecular fingerprinting techniques, research on the canine GI microbiome revealed that differences in the levels of canine GI microbial diversity existed between individuals and intestinal compartments.

Effect of diets and nutrients on the canine gut microbiota

Compared with environmental factors, diet plays an important role in adjusting the GI microbiome balance in canines. Next-generation sequencing (NGS) has revolutionized the approaches in researching complex microbiota samples. Fecal samples from canine models can be analyzed via NGS to determine the relationships and interactions between hosts and the microbiota.[12]

High-throughput sequencing has also revealed interactions between the canine gut microbiota and the diet. Different ratios of proteins and carbohydrates, prebiotics, and dietary habits are the major research directions for the canine gut microbiota. Inulin and other fibers are current research topics on the gut microbiota.[13] One study revealed that consuming a small amount of beet pulp as dietary fiber changed the abundance of the typical canine gut microbiome. Adding 7.5% beet pulp (60% of the total dietary fiber) to standard canine diets for 14 days significantly shifted the Firmicutes abundance and tripled the low taxonomic levels of *Faecalibacterium*. *Eubacterium hallii*, which produces butyrate, appeared after a fiber diet treatment.[14] These abundance changes may have been due to dietary selection for complex fermentative activity with additional dietary fiber. In another study, overweight canines were fed an approximately 1% inulin-type prebiotic diet, and the results showed that inulin-type prebiotics may modulate the gut microbiota, short-chain fatty acid (SCFAs) and bile acids (BAs) in overweight dogs. The overweight canines had significantly increased SCFAs and lower indole concentrations after prebiotic treatment. Fermentation of inulin-type prebiotics produced SCFAs, which decreased the luminal pH and prevented BA reabsorption. BAs are the major pathway for excreting cholesterol from the body.[15] Another study showed that continuously feeding dogs low-fat and high crude-fiber diets for 17 weeks decreased the body weights of obese dogs by an average of 18% of the initial body weight.[16]

Thus, inulin-type prebiotics and fibers can modulate canine fecal metabolites such as SCFAs and BAs. Bacterial diversity can also be changed with prebiotic and fiber treatment. Studies on body weight loss in canines also indicate that prebiotics and fiber may play important roles in improving SCFA production, body conditions and other potential metabolic factors.

Different formulated commercial foods and natural foods with different nutrient sources and ratios (such as proteins and carbohydrates) are widely used in canine diets. Daily diets with different ingredients also alter the abundance and diversity of the canine gut microbiota. High-protein, low-carbohydrate (HP-LC) diets can modulate body weight in obese canines.[17] Canine models fed HP-LC diets had lower Bacteroidetes/Firmicutes ratios and higher Bacteroides/Prevotella ratios than did those fed low-protein HC diets, and the abundances of *Clostridium hiranonis*, *Ruminococcus gnavus*, and *Clostridium perfringens* were increased. Enriched microbial gene networks are also associated with weight maintenance.[18]

Another study showed that HP diets increased the abundance of butyrate-producing bacteria and enhanced the activity in canine gut microbiomes independent of body condition. Canines fed HP diets had higher concentrations of fecal ammonia, isovalerate, isobutyrate, phenol, indole, serum indoxyl sulfate and plasma 3-OH isovalerylcarnitine, and these dogs had diarrhea. High concentrations of these metabolites are correlated with high protein intake.[19–21] Compared with lean canines, increased dietary protein content more strongly affected the gut microbiotas in obese canines.[22] No differences were found in gut bacterial richness between canines fed high-fat, low-starch and low-fat, and high-starch diets. However, lower relative abundances of *Prevotella*, *Solobacterium*, and *Coprobacillus* were found in the high-fat diet group as well as higher concentrations of fecal BAs after 7 weeks of feeding. Moderate variations in these diets did not alter the dogs' gut microbiotas.[23]

One study replaced protein and starch with navy beans in canine diets to investigate the effect of different nutrient sources on canines. In the navy bean diet, which included 25% navy bean powder, macronutrients, and micronutrients, the total calories were adjusted to match the standard diet. After feeding canines the navy bean or standard diet for 4 weeks, the gut microbial populations did not significantly differ between dogs fed either diet. Compared with the baseline, the navy bean-diet group had increased Firmicutes and decreased Actinobacteria and Fusobacteria.[24]

Dietary models with different nutritional concentrations have revealed that macronutrient ratios are key factors in modulating the canine gut microbiota (Table 1). Distinct bacterial diversity and populations can be observed in the gut microbiotas and fecal metabolites of dogs fed different diets. If different diets contain the same levels of macronutrients and micronutrients, the effect on the bacterial population in the canine gut microbiota may be minimal.

Feeding dogs natural vs commercial foods also affects the microbiota population and abundance. Meat is the major protein supplement in natural dog foods. One study fed dogs natural and commercial diets for 1 year and revealed biases in the microbial abundances and populations in the gut microbiota. Canine natural diets containing approximately 90% raw meat were divided into 4 groups with different meats: kangaroo, beef, chicken and duck. The other two groups were supplemented with commercial foods with balanced nutrition. Despite the various meat sources, all canines fed natural diets had higher gut microbiome diversity than did the commercial-feed groups. Beta diversity analysis showed that the canine gut microbiota diversity differed significantly between the dietary types. *Clostridium perfringens* and *Fusobacterium varium* in the canine gut microbiota were more abundant in dogs fed the natural diets. In this study, the natural diet had 30–52% crude protein and 11–50% total fat content regardless of meat type, while the commercial canine foods had 18–21% crude protein and 8–10% total fat content.[25] Another study of the canine gut microbiota compared the interaction between dogs fed a canine commercial extruded diet and dogs fed a mixed natural diet with 70% beef. After feeding for 14 days, the diets of the two groups were reversed, and the dogs were fed for another 14 days, but dogs fed the commercial extruded diet were shifted to the natural diet and vice versa. The dietary interactions showed that the natural diet promoted more balanced bacterial growth in the canine gut

Table 1. Diet-related studies of the canine gut microbiota and metabolites.

Samples	Experimental design & Period	Target factor	Method	Alteration in experimental group	Reference
Fecal (n = 6)	Control diet(30% CP, 20% fat) vs Fiber diet(control diet with 7.5% beet pulp); Two 14-d periods; Healthy canines	Dietary fiber	454-Pyrosequencing of 16S rRNA gene (V3 region)	Microbiome: (in fiber diet group) ↑: Firmicutes, Clostridia ↓: Fusobacteria, Actinobacteria, Erysipelotrichi	Middelbos et al.,[14]
Fecal (n = 9)	Control diet(38% CP, 17% fat) with placebo vs 0.5% vs 1% inulin-type fructans; Three 14-d periods; OW canines	Prebiotic	Illumina MiSeq sequencing of 16S rRNA gene (V4 region); qPCR; Gas Chromatography	Microbiome: (in 1% group) ↑: Eubacterium, Turicibacter, Erysipelotrichaceae ↓: Coprobacillus, Anaerobiospirillum Fecal Metabolites: (in 1% group) ↑: Total SCFA, acetate, butyrate, propionate, valerate, deoxycholic acid (DCA), lithocholic acid (LCA) and total BA ↓: Indole (numerically lower, not significant) Other: (in 1% group) ↓: Fecal dry matter percentage	Alexander et al.,[15]
Fecal & Serum (n = 6)	Control diet(28% CP, 15% fat, 29% starch, 2% crude fiber) for adaptation, then give hypocaloric diet(33% CP, 8% fat, 20% starch, 10% crude fiber) for body weight loss program; 17-week period for body weight loss program; OW canines	Dietary fiber; Body weight	Illumina MiSe sequencing of metagenomics; Serum biochemistry analysis	Microbiome: (in hypocaloric diet group) ↑: Allobaculum and greater biodiversity ↓: Clostridium, Lactobacillus, Dorea Serum Metabolites: (in hypocaloric diet group) ↓: Cholesterol, alkaline phosphatase, paraoxonase 1 (PON1) Other: ↓: Approximate 18% body weight loss at the end	Salas-Mani et al.,[16]
Fecal (n = 64)	HPLC diet(49% protein, 11% carbohydrate) vs LPHC diet(26% protein, 39% carbohydrate); Two 4-week periods; 32 healthy canines and 32 OW canines	Protein & Carbohydrate; Body weight	Illumina MiSeq sequencing of 16S rRNA gene (V3-V4 regions)	Microbiome: (in HPLC diet group) ↑: Clostridiaceae, Lachnospiraceae, Ruminococcaceae, Vagococcus, Streptococcus, Clostridium perfringens, Clostridium hiranonis, Ruminococcus gnavus ↓: Fusobacterium, Turicibacter,Parabacteroides, Prevotella, Erysipelotrichaceae, Veillonellaceae, Lactobacillaceae, Bacteroides uniformis, Clostridium butyricum (↓Prevotella copri was only observed in OW canines) Others: LPHC diet group had higher Bacteroidetes-to-Firmicutes ratios (B/F ratios) in OW canines, the ratios diminished in healthy canines HPLC diet group had higher Bacteroides-to-Prevotella ratios (B/P ratios) in both healthy and OW canines	Li et al.,[18]
Fecal & Serum (n = 10)	Two isocaloric diet: HP diet(50% CP) vs LP diet(18% CP); Two 4-week periods; 6 healthy canines and 4 OW canines	Protein Body weight	DGGE & qPCR; Gas Chromatography; Serum biochemistry analysis	Microbiome: (in HP diet group) ↑: Healthy canines had higher abundance of Firmicutes, Lactobacillus, clostridial cluster I than obese canines when comparing with LP diet Fecal Metabolites: (in HP diet group) ↑: Ammonia, isovalerate, isobutyrate, phenol, indole Serum Metabolites: (in HP diet group) ↑: Indoxyl sulfate, 3-OH isovalerylcarnitine	Xu et al.,[22]

(Continued)

Table 1. (Continued).

Samples	Experimental design & Period	Target factor	Method	Alteration in experimental group	Reference
Fecal & Serum (n = 12)	LFHS diet(10% fat, 44% starch) vs HFLS diet(21% fat, 27% starch); 7-week period and 2-week washout period; Healthy canines	Fat & Starch	Ion-Torrent sequencing of 16S rRNA gene (V1-V2 regions); Kinetic chromogenic assay (Measurement of serum LPS)	Microbiome: (in HFLS diet group) ↑: Megamonas ↓: Prevotella, Solobacterium, Coprobacillus Fecal Metabolites: (in HFLS diet group) ↑: Total BA, glycochenodeoxycholic acid (GCDCA), taurocholic acid (TCA), deoxycholic acid (DCA) Serum Metabolites: LPS had no significant changes in both group	Schauf et al.,[23]
Fecal (n = 21)	Control diet(30% CP, 14% fat) vs Navy bean diet(included 25% cooked navy beans with equal macronutrient, micronutrient and total caloric content); 4-week period; Healthy canines	Nutrient source	454-Pyrosequencing of 16S rRNA gene (V4–V6 regions)	Microbiome: No significant alterations were observed in both group Navy bean diet group had ↑Firmicutes, ↓Actinobacteria, Fusobacteria after treatment compared to baseline	Kerr et al.,[24]
Fecal (n = 11)	Natural diet(90% raw meat, 10% vegetable) vs commercial diet (18–21% CP, 8–10% fat); At least 1-year period; Healthy canines	Dietary	Illumina MiSeq sequencing of 16S rRNA gene (V3-V4 regions)	Microbiome: (in natural diet group) ↑: Fusobacteria, Fusobacteriaceae, Fusobacterium varium, Clostridium perfringens, Clostridium rectum, Clostridium hiranonis, Clostridium sordelli, Clostridium glycyrrhizinilyticum, Eubacterium tenue, Eubacterium dolichum and greater biodiversity Microbiome: (in commercial diet group): ↑: Coprobacillus, Clostridium ramosum, Anaerostipes caccae,	Kim et al.,[25]
Fecal (n = 8)	Raw meat diet(with 70% beef) vs commercial diet(26% CP, 11% fat), after first period, two groups exchanged the diet for each other; Two 2-week periods; Healthy canines	Dietary	Illumina MiSeq sequencing of 16S rRNA gene (V3-V4 regions); High Performance Liquid Chromatography(HPLC)	Microbiome: (in raw meat diet group) ↑: Clostridium XI, Clostridium XIX, Bacteroides, Fusobacterium, Cetobacterium, Escherichia/Sighella, Lactococcus and greater biodiversity ↓: Lactobacillus, Paralactobacillus, Prevotella Fecal Metabolites: (in raw meat diet group) ↑: lactic acid ↓: acetic acid Other: Fecal butyrate was positively correlated with Blautia and Peptococcus Fecal acetate was negatively correlated with Escherichia/Shigella and Megamonas	Sandri et al.,[26]

(Continued)

Table 1. (Continued).

Samples	Experimental design & Period	Target factor	Method	Alteration in experimental group	Reference
Fecal (n = 11)	Control diet(CD1, 27% CP) vs meat diet (control diet with increments of 25%, 50% and 75% beef), after given meat diet, all canines were given control diet again(CD2) for 2 weeks again; 7-week period; Healthy canines	Dietary	Illumina MiSeq sequencing of 16S rRNA gene (V3-V4 regions); Gas Chromatography	Microbiome: (in meat diet group with 75% beef, comparing with CD1) ↑: *Clostridiaceae, Clostridiaceae hiranonis, Coriobacteriales, Coriobacteriaceae, Dorea, Slackia* ↓: *Faecalibacterium*, lower biodiversity Microbiome: (in meat diet group with 75% beef, comparing with CD2) ↑: *Clostridiaceae, Clostridiacea hiranonis, Erysipelotrichaceae, Roseburia, Dorea, Slackia,* ↓: *Faecalibacterium, Veillonellaceae* Fecal Metabolites: (in meat diet group with 75% beef, comparing with CD2) ↑: Butyric acid ↓: Acetic acid, isovaleric acid Other: ↑: Fecal pH in 75% meat diet group	Herstad et al.,[27]
Fecal (n = 15)	Control diet(30% CP and 27% fat in DM) vs meat diet(76% CP, 18% fat in DM); 9-week period; Healthy canines	Dietary	454-Pyrosequencing of 16S rRNA gene (V4-V6 regions); Gas Chromatography	Microbiota: (in meat diet group) ↑: *Clostridium, Fusobacterium, Lactobacillus* ↓: *Bacteriodes, Faecalibacterium, Peptostreptococcus, Prevotella* Fecal Metabolites: (in meat diet group) ↓: Acetate, propionate, butyrate, total VFA, isobutyrate, valerate, isovalerate	Bermingham et al.,[28]

↑: increased; ↓: decreased; rRNA: ribosomal RNA; OW: overweight; CP: crude protein; HP/LP: high/low protein; HC/LC: high/low carbohydrate; HF/LF: high/low fat; HS/LS: high/low starch; DM: dry matter

microbiota.[26] Similar research showed that changing from commercial dry food to a natural diet with minced beef influenced the canine gut microbiome. The concentration of the minced beef in the natural diet was increased from 25% to 75% of the dogs' total energy requirement in 3 weeks. The results showed that the changes in the canine gut microbiome were reversible. The minced beef diet influenced the fecal microbiota concentration and SCFA profiles in the canine fecal samples, and the isovaleric acid concentration increased as the protein consumption increased. Recent research on the human microbiota revealed that higher concentrations of fecal isovaleric acid were correlated with depression.[29] Reintroducing the dogs to the initial commercial dry food significantly reversed these changes.[27] The differences in protein and energy digestion in canines revealed that the key bacterial families, Clostridiaceae, Erysipelotrichaceae and Bacteroidaceae, played important roles in the relationships between the canine gut microbiota, nutrient digestibility and metabolism.

In another study, dogs were divided and fed kibble or meat diets. The correlation heatmap and relevance network plot showed that Clostridiaceae was the central node in the canine gut microbiota, nutrient digestibility and macronutrient composition. Clostridiaceae, dietary protein content, and protein digestibility were positively correlated, while fecal protein content was negatively correlated with Clostridiaceae. The Clostridiaceae abundance also increased when dogs were fed a meat diet.[28] Erysipelotrichaceae are important bacteria in the canine digestive tract and are associated with high-fat diets in humans and rodent models.[30–32] Erysipelotrichaceae were positively correlated with markers associated with carbohydrate digestion, such as dietary carbohydrates, fiber content and volatile fatty acid (VFA) production. In addition, Erysipelotrichaceae were negatively correlated with protein metabolism markers. Bacteroidaceae, as with other members of Bacteriodetes, played a role in nutrient digestion in canines, producing several VFAs, including succinate, acetate and propionate. Propionate is produced from succinate,[33] and acetate and propionate can activate both GPR41 and GPR43. GPR41 and GPR43 are a pair of mammalian G protein-coupled receptors thought to be potential targets for metabolic disorders such as obesity.[34,35] Clostridiaceae, Erysipelotrichaceae and Bacteroidaceae were at the center of the relationship between the canine gut microbiota and nutrient digestibility, and changes in the canine gut microbiota were related to diet. Canines fed natural diets had more complex bacterial populations in their gut microbiotas than did those fed formulated commercial diets. Thus, differences in macronutrients between natural diets and commercial food strongly suggest that nutrients affect the canine gut microbiota. The canine gut microbiota also affects nutrient digestibility, and some bacteria appear more important in modulating the microbiome. Significant changes in the canine gut microbiota have been observed with both short-term and long-term feeding, and reversibility exists with short-term feeding. Canine fecal metabolites also differ between dogs fed natural food versus commercial food. Although diets with different macronutrient ratios exert significantly different effects on the canine gut microbiota, the effect of micronutrients in natural foods as well as whether micronutrients play specific roles in modulating the canine gut microbiota remains uncertain.

Canine gut microbiota and GI diseases

Inflammatory bowel disease (IBD) refers to a diverse group of chronic gastrointestinal diseases.[36] IBD is an autoimmune condition that is difficult to diagnose, and gut microbiome dysbiosis has been proposed as an induced factor in its pathogenesis.[37] Dysbiosis of the human gut microbiome exerts similar changes to those seen with gut dysbiosis in canines and other mammals. Research on canine IBD is valuable because it enables making predictions from the canine gut microbiota to the human gut microbiota.[38] Here, we discuss the relationships between the canine gut microbiota, metabolism, other factors in canine IBD, and other GI diseases. Table 2 summarizes some recent studies of GI diseases and the canine gut microbiota.

One study proposed that microbiotas enriched with Gram-negative bacteria in obese canines may be correlated with chronic inflammation. These

Table 2. GI disease-related studies of the canine gut microbiota.

Disease	Method	Samples	Alteration in GI-diseases group	Reference
IBD		Fecal	**Microbiome:**	
	454-pyrosequencing of the 16S rRNA gene (V1-V3 regions) & qPCR	n=19	↓: Fusobacteria, *Faecalibacterium* spp.	Suchodolski et al., 2012[40]
	qPCR	n=23	No differences were found between healthy canines and IBD canines	Xu et al., 2016[42]
	454-pyrosequencing of the 16S rRNA gene (V4-V6 regions) & qPCR	n=12	↑: Gammaproteobacteria, ↓: Erysipelotrichia, Clostridia, Bacteroidia and lower biodiversity	Minamoto et al., 2015[54]
			Fecal Metabolites:	
	Gas chromatography (GC)	n=23	↑: 3-hydroxyisovalerylcarnitine + isovalerylcarnitine to leucine ratios ↓: Short-chain acylcarnitines to free carnitine ratios	Xu et al., 2016[42]
		Blood	**Serum Metabolites:**	
	Serum biochemistry analysis	n=23	↑: Valine ↓: Citrulline, folate	Xu et al., 2016[42]
	GC-TOF/MS	n=12	↑: 3-hydroxybutyrate, hexuronic acid, ribose, gluconic acid lactone	Minamoto et al., 2015[54]
CE		Fecal	**Microbiome:**	
	Illumina MiSeq sequencing of 16S rRNA gene (V4-V6 regions) & qPCR	n=73	↑: *Bifidobacterium* spp., *Lactobacillus* spp., *Streptococcus* spp., *Escherichia coli* ↓: Bacteroidetes, *Blautia* spp., *Clostridium hiranonis*, *Faecalibacterium* spp., *Fusobaterium* spp., *Turicibacter* spp. and lower biodiversity	Minamoto et al., 2019[57]
	qPCR	n=106	↑: *Streptococcus*, *Escherichia coli* ↓: *Blautia*, *Clostridium hiranonis*, *Faecalibacterium*, *Turicibacter*, *Fusobacterium* and total bacteria	AlShawaqfeh et al., 2017[60]
			Fecal Metabolites:	
	Gas chromatography-mass spectrometry (GC-MS)	n=73	↓: Total SCFA, acetate, propionate	Minamoto et al., 2019[57]
NHD		Fecal	**Microbiome:**	
	454-pyrosequencing of the 16S rRNA gene (V1-V3 regions) & qPCR	n=12	↓: Ruminococcaceae, *Blautia* spp.	Suchodolski et al., 2012[40]
AHD		Fecal	**Microbiome:**	
	454-pyrosequencing of the 16S rRNA gene (V1-V3 regions) & qPCR	n=13	↑: *Sutterella*, *Clostridium perfringens* ↓: *Blautia*, Ruminococcaceae, *Faecalibacterium*, *Turicibacter* spp.	Suchodolski et al., 2012[40]
	qPCR	n=7	↓: Ruminococcaceae	Heilmann et al., 2017[59]
			Fecal markers of inflammation:	
	Immunoassays	n=7	↑: Calprotectin, S100A12, α$_1$-proteinase inhibitor	Heilmann et al., 2017[59]
AD		Fecal	**Microbiome:**	
	454-pyrosequencing of the 16S rRNA gene (V4-V6 regions) & qPCR	n=13	↑: *Clostridium* ↓: Bacteroidetes, *Faecalibacterium*, Ruminococcaceae and lower biodiversity	Guard et al., 2015[58]
			Fecal Metabolites:	
	UPLC-MS & HPLC-MS	n=13	↓: Propionic acid	Guard et al., 2015[58]
		Blood	**Serum Metabolites:**	
	Serum biochemistry analysis	n=13	↓: Kynurenic acid, kynurenic to tryptophan ratios	Guard et al., 2015[58]

↑: increased; ↓: decreased; IBD: inflammatory bowel disease; CE: chronic enteropathy; NHD: acute non-hemorrhagic diarrhea; AHD: acute hemorrhagic diarrhea; AD: acute diarrhea (both NHD and AHD combined); qPCR: quantitative PCR; GC-TOF/MS: gas chromatography coupled with time-of-flight mass spectrometry; UPLC-MS: ultra-performance liquid chromatography-mass spectrometry; HPLC-MS: high-performance liquid chromatography-mass spectrometry

authors fed the same diet to dogs for 6 months. Canines in the obese group were fed food ad libitum, while dogs in the lean group were fed food with restricted nutrients. The obese group had significantly increased body weight, higher leptin concentrations, and decreased adiponectin and 5-hydroytryptamine (5-HT) in the cerebrospinal fluid. Proteobacteria were predominant in the gut microbiotas of the obese canines (76%), while Firmicutes (85%) were predominant in the lean canines. Enriched Gram-negative bacteria can influence intestinal lipopolysaccharide (LPS) levels. Decreased 5-HT concentrations in obese canines can increase their appetites and cause a cycle leading to bacterial dysbiosis with increased Gram-negative bacteria and enriched LPS. LPS is associated with

chronic inflammation.[46] This research revealed the relationships between IBD, diet and bacterial dysbiosis.

Another study characterized the bacterial dysbiosis among canine idiopathic IBD, acute non-hemorrhagic diarrhea and acute hemorrhagic diarrhea (AHD). Compared with healthy dogs, those with AHD had the most significant changes in gut microbiota abundance and diversity. The abundances of *Faecalibacterium, Ruminococcaceae, Turicibacter* spp. and *Blautia* spp. were significantly decreased, while *Sutterella* and *Clostridium perfringens* were significantly increased. qPCR assay results confirmed that only canines with AHD had significant increases in *Clostridium perfringens*. Some populations of decreased bacteria were believed to be important producers of SCFAs. *Faecalibacterium* spp. and *Fusobacteria* were decreased in dogs with active IBD. *Faecalibacterium* spp. were decreased in the canine gut microbiotas in dogs with both acute diarrhea and IBD. Decreases in *Faecalibacterium prausnitzii* often occur in human IBD.[39] Studies have shown that *Faecalibacterium prausnitzii* secretes anti-inflammatory peptides *in vitro*,[47] which may demonstrate the relationships between GI disorders and microbiota dysbiosis. IBD influenced the canine gut microbiota profiles and metabolism.

The canine chronic enteropathy clinical activity index (CCECAI) is linked with the clinical scores of canines with IBD. In this study, the total CCECAI score was divided into 5 levels by IBD symptoms as follows: clinically insignificant (0–3), mild (4–5), moderate (6–8), severe (9–11), or very severe (≥12). CCECAI was significantly negatively correlated with the total fecal SCFA concentration and the *Lactobacillus* abundance in the canine gut microbiome. Dogs characterized as having severe IBD had low proportions of *Lactobacillus*, higher plasma concentrations of valine, free carnitine and total acylcarnitines, decreased plasma concentrations of citrulline and short-chain acylcarnitine-to-free carnitine ratios and decreased folate in the serum. Canines with IBD also had higher 3-hydroxyisovalerylcarnitine concentrations and isovalerylcarnitine/leucine ratios compared with those of the healthy group, and these increased levels may be associated with the catabolism of branched-chain amino acids.[40] Valine has been implicated in lipid and fatty acid metabolism.[48,49] The metabolism of carnitine in the gut microbiota

can produce trimethylamine (TMA), and further metabolism of TMA to trimethylamine-N-oxide (TMAO) can accelerate atherosclerosis.[50] Human studies have shown that acylcarnitines are associated with insulin resistance,[51,52] and short-chain acylcarnitine can be hydrolyzed to carnitine.[53] Citrulline is an amino acid and validated marker of enterocyte function and the gastrointestinal barrier, which is produced by enterocytes of the small bowel mucosa. Decreased concentrations of citrulline can reduce the enterocyte mass such as that in short bowel syndrome.[54] Accumulations of 3-hydroxyisovalerylcarnitine and isovalerylcarnitine in higher concentrations are associated with metabolic deficiency in leucine metabolism and with protein absorption from foods.[55–57] Lower folate concentrations in the serum are linked to abnormal functioning of the proximal small intestinal mucosa, which can reduce folate absorption.[58] Tissue damage and alterations in the host metabolism can significantly change the short-chain acylcarnitines, amino acids in the plasma, and other metabolic profiles in canines with IBD.[40] Further evidence showed dysbiosis of other metabolites and canine gut microbes in canines with IBD. Compared with healthy canines, canines with IBD had significantly lower gut microbiota diversity. Canine serum metabolites also differed significantly. Canines with IBD had higher concentrations of 3-hydroxybutyrate, hexuronic acid, ribose, and gluconic acid lactone than did healthy canines.[41] The higher concentrations in the ketone body of 3-hydroxybutyrate may be correlated with a high energy demand in the host.[59] Hexuronic acid is also known as vitamin C and is considered an antioxidant because it protects against oxidative stress and plays an important role in collagen and carnitine synthesis.[60] Ribose is required for biological systems and provides further metabolism. Gluconic acid lactone and ribose both protect the cells from oxidative stress.[41] Although clinical symptoms of canine IBD were improved after medical therapy, no significant changes occurred in the serum metabolite profiles or gut microbiotas. Enriched bacterial functions of the secretory system, transcription factors and an unknown pathway were overrepresented in canines with IBD, while amino acid metabolism was enriched in healthy canines. Canines with IBD and other GI diseases showed gut microbiota dysbiosis and altered indexes, such as 5-HT, in the serum. Dysbiosis was also found in the

fecal SCFA concentrations in canines with IBD; thus, a model of SCFA dysbiosis can be used to evaluate the clinical symptoms of canine IBD.

Canines with chronic enteropathy had symptoms of SCFA dysbiosis. Research on 49 healthy canines and 73 canines with chronic enteropathy showed higher total SCFA concentrations in healthy canines, while canines with chronic enteropathy had lower acetate and propionate concentrations. Higher dysbiosis indices and decreased bacterial diversity and richness were observed in the gut microbiotas of canines with chronic enteropathy,[42] thus revealing the clinical importance of canines with chronic enteropathy having altered concentrations of fecal SCFAs and gut microbes. Microbiota dysbiosis and other symptoms also existed in canines with acute diarrhea (AD). Evaluations of the gut microbiota, fecal SCFAs, serum, and urine metabolite profiles revealed differences between healthy canines and canines with AD. Canines with AD also presented significantly lower bacterial diversity and altered microbial communities with different representative bacteria. *Clostridium* was significantly increased in canines with AD. Fecal propionic acid decreased and was associated with decreased *Faecalibacterium* in canines with AD. Total fecal SCFAs and branched-chain fatty acids showed no significant changes. The predicted functional gene content of the microbiome also revealed that genes for the methyl-accepting chemotaxis protein and the transposase enzyme were overrepresented. Serum kynurenic acid concentrations were significantly decreased in canines with AD. Urine concentrations of 2-methyl-1H-indole and 5-methoxy-1H-indole-3-carbaldehyde were also significantly decreased in dogs with AD.[45]

Further research associated canines with idiopathic acute hemorrhagic diarrhea syndrome (AHDS) with fecal markers and microbiota. Idiopathic AHDS is characterized by acute onset of bloody diarrhea and severe dehydration and can result in high mortality rates if left untreated. In this study, 3 assayed markers were selected to evaluate idiopathic AHDS. Calprotectin and S100A12 were the markers of inflammation, and α_1-proteinase inhibitor was the marker of gastrointestinal protein loss. All 3 markers had significantly higher concentrations in canines with idiopathic AHDS. After 3 days of treatment, these markers decreased significantly.[44] Canine gut microbiota dysbiosis appeared to last longer than did the markers of inflammation and protein loss. Canines with AHDS had gut microbiota dysbiosis and abundances of specific bacteria, including *Ruminococcaceae*, *Faecalibacterium* spp., *Bifidobacterium* spp., and *Proteobacteria*. This microbiota dysbiosis did not resolve immediately after treatment. Bacterial diversity did not differ in the canine gut microbiota except a mild decrease in *Ruminococcaceae* 3 days after treatment.

Another study using qPCR established a mathematical model termed the dysbiosis index (DI) in canine chronic inflammatory enteropathy (CE) to evaluate the gut microbiota dysbiosis level. Eight indexes of the universal bacteria, *Faecalibacterium*, *Turicibacter*, *Streptococcus*, *Escherichia coli*, *Blautia*, *Fusobacterium*, and *Clostridium hiranonis*, were measured via qPCR to assess the DI. The DI in canines with different CE levels was defined as a mathematical formula using these 8 indexes to compare the bacteria with those of healthy canines.[43] Canines with CE differed significantly from healthy canines in all 8 bacterial target indexes. *Escherichia coli* and *Streptococcus* were significantly higher in the diseased group, while the other indexes were significantly lower. The CCECAI was significantly correlated with DI. qPCR-based indexes of this model showed that the model had a 74% sensitivity and 95% specificity to separate CE canines and healthy canines. This model also provided a new approach to assess whether the canine gut microbiota was normalized in response to CE treatment. In canines with other GI diseases, SCFA dysbiosis and gut microbiota dysbiosis are common. Therapy for GI diseases shows that the canine gut microbiota does not return to normal shortly after treatment, and a canine GI disease model can be established and evaluated using specific bacteria in the canine gut microbiota.

Canine gut microbiota dysbiosis often occurs with canine GI diseases. Treatments for GI diseases that modulate the gut microbiota, such as probiotic therapy and fecal microbiota transplantation, have been used to treat some human GI diseases, and researchers have asked whether similar therapies can be used to treat canine GI diseases.

Probiotic-related studies in canines

Probiotic therapy has been used in human GI diseases and may have potential use in treating canine GI diseases.[61] Canine-derived probiotics for treating canine GI disease comply with ethical standards and have been shown to have health benefits in treating canine GI diseases. One study revealed that canine-derived *Lactobacillus fermentum* CCM 7421 significantly modulated canine diarrhea. Administering 10^7–10^9 colony-forming units of the probiotic, *Lactobacillus fermentum*, to canines for 4–14 days normalized canine metabolites, including SCFAs. Using probiotic strains also modulated serum biochemical and immune parameters regardless of the duration of administration. *Clostridia* populations in the gut microbiotas of dogs with diarrhea were decreased, and lactic acid bacteria were increased after treatment.[62]

Another study evaluated standard therapy and standard therapy + multi-strain probiotics on the mucosal microbiota for 8 weeks in canines with IBD. The probiotic strains, *Lactobacillus plantarum* DSM 24730, *Streptococcus thermophiles* DSM 24731, *Bifidobacterium breve* DSM 24732, *Lactobacillus paracasei* DSM 24733, *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 24734, *Lactobacillus acidophilus* DSM 24735, *Bifidobacterium longum* DSM 24736, and *Bifidobacterium infantis* DSM 24737, were combined in specific concentrations. Fluorescence in situ hybridization methods showed that the mucosal microbiotas from canines with IBD in the standard therapy and standard therapy + probiotics groups did not significantly differ in bacterial numbers. Both treatments increased the mucosal bacterial species and total bacterial numbers. Standard therapy showed increased *Bifidobacterium* spp., while standard therapy + mixed probiotics showed increased *Lactobacillus* spp. in the canine mucosal microbiota. Canines with IBD in the standard therapy + probiotic group had increased tight-junction protein expression. The probiotic therapy was also associated with upregulated expression of the tight-junction proteins, E-cadherin, occludin, and zonulin,[63] showing that probiotic therapy may benefit mucosal homeostasis.

Other probiotic strains can also modulate the composition and function of the gut microbiota in canines with recurrent diarrhea. The probiotic strains, *Lactobacillus casei* Zhang, *Lactobacillus plantarum* P-8, and *Bifidobacterium animalis* subsp. *lactis* V9, were mixed. Canines with recurrent diarrhea were given probiotic therapy for 60 days, and fecal samples were analyzed using metagenomics. After probiotic therapy, diversity and bacterial populations in the canine gut microbiota were significantly altered. Beneficial bacteria were increased in the canine gut microbiota. The abundances of *Lactobacillus acidophilus*, *Lactobacillus johnsonii*, *Lactobacillus reuteri* and *Butyricicoccus pullicaecorum* were significantly increased, and opportunistic pathogenic bacteria, such as *Clostridium perfringens* and *Stenotrophomonas maltophilia*, were significantly decreased. Metagenomic analysis revealed that probiotic therapy was significantly associated with upregulated pathways related to amino acid metabolism, metabolism of cofactors and vitamins, biosynthesis of secondary metabolites, and glycan biosynthesis and metabolism. Pathways associated with virulence of pathogenic bacteria and cell signaling were downregulated after probiotic therapy.[64] Therefore, probiotic therapy may play an important role in treating canine GI diseases.

Isolating new probiotic strains from canine feces is also important. Culturing potential probiotic strains may promote canine health. One study isolated a series of potential probiotic strains of *Lactobacillus* from canine fecal samples, and the effects were evaluated. Properties of the strains, including resistance to gastric and pancreatic juices and bile salts, antibiotic resistance and antipathogenicity, were tested. Of the 14 isolated *Lactobacillus* strains, 5 could tolerate gastric stress. These 5 potential probiotic strains also had clindamycin resistance and significant antimicrobial capacity in inhibiting pathogenic strains. Morphological and molecular characterization identified that 3 of these strains were *Lactobacillus reuteri*, and the other two were *Lactobacillus johnsonii*. Identification of *Lactobacillus reuteri* in vitro confirmed its ability to ferment sugar and adhere to HT29 epithelial cells; thus, *Lactobacillus reuteri* may be a potential probiotic.[65]

Isolating potential new probiotic strains is crucial for probiotic therapy. Culturing new bacterial isolates is a research frontier in the human gut microbiota, and probiotic strains isolated from the human gut have played important roles in

modulating the gut microbiome balance. Probiotic isolation and therapy could become a new method of treating canine GI diseases.

Similarities between the canine and human gut microbiotas

A recent metagenomic study reported that in dogs who randomly received either an HP or HC diet, the canine microbiome diversity was closer to that of the human microbiome than to the microbiomes of either pigs or mice.[66] Factors including breed and sex had little influence on the gut microbiome changes. As the results were consistent with those of previous human studies, canine studies may predict results in humans. Other studies on canines validate this conclusion.

Dogs are the oldest known example of domestication. Domestication of canines from wolves is a key factor influencing the microbiota diversity between domesticated canines and wolves. Of six bacterial genera isolated from canine fecal samples, *Fusobacterium* and *Ruminococcus gnavus* are typical inhabitants of canine guts, whereas these two genera are not represented in the healthy human gut microbiota. Compared with wild wolves, canine domestication seems to have caused the loss of these six bacterial genera in canines.[67] Interestingly, domesticated canines acquired five bacterial taxa that exist in the healthy human core gut microbiota. The *Bifidobacterium*

population in the domesticated canine gut microbiota showed a higher average relative abundance and diversity in species numbers than those of nondomesticated canines. Hence, these results revealed that under the circumstances of cohabitation with humans, the canine gut microbiota coevolved with that of its host. Canines can adapt and gain resilience against dietary changes, and this resilience is also seen in the human gut microbiota.[68] Domestic canines had several microbial taxa related to cellulose and starch digestion. Metagenomics showed that the metabolic pathways of domestic canines and wolves also differed in the abundances of encoding genes, and significant differences existed in genes encoding glycosyltransferase family 34 (GT34), carbohydrate-binding module family 25 (CBM25), and glycoside hydrolase family 13 (GH13) between domestic canines and wolves. Such differences also occurred in carbohydrate enzymes, especially amylose, sucrose, and maltose. Compared with other mammals, domestic canines are more similar to humans in their gut microbiota, and consuming similar diets to those of humans played an important role in canine domestication.[69]

Further research on canine domestication provides additional evidence of the similarities in the gut microbiota between canines and humans. Population genetics analysis shows that humans and canines both experienced similar environments during canine domestication (Figure 1).
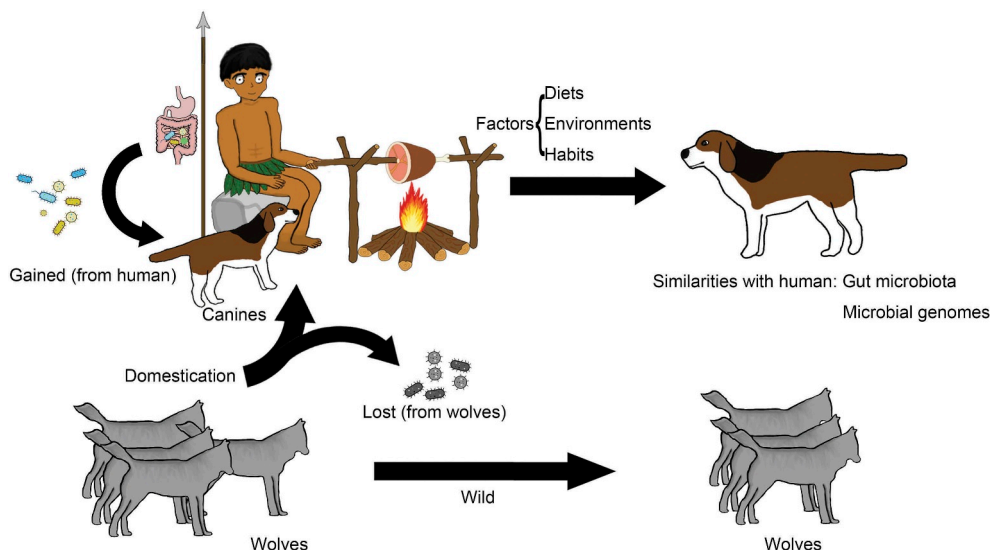

Figure 1. Domestication played an important role in altering the canine gut microbiota. Canine domestication led to the loss of some gut bacteria compared with those of nondomesticated wolves, while interaction with humans resulted in new gastrointestinal bacteria in canines.

Natural selection, driven by convergent environmental pressures, may have played an important role in modulating a similar set of genes between the two genomes of humans and canines. Environmental factors might have driven positive selection of some genes. A group of genes related to digestion and metabolism in both species appear to have been under positive selection during domestication. Many positive selections in both species show parallel evolution. Another interesting conclusion is that positive selection acted on genes involved in neurological processes in both humans and canines. The genes involved in canine neurological processes overlap extensively with these genes in humans.[70] The close interactions between humans and canines as well as drastic changes in the canines' living environments may have caused some of the striking parallelism between human and canine genetic evolution. These interesting conclusions may provide potential approaches in using canine models to investigate neurological diseases.

Research on the gut-brain axis has shown that the gut microbiota is significantly correlated with neurological diseases. The gut-brain axis presents new opportunities for medical and probiotic therapies in neurogastroenterology.[71] However, few articles to date have reported the impacts of the canine gut microbiota on the gut-brain axis and neurological diseases in canine models.

Some research on human GI diseases has been based on rodent models, which have provided meaningful results regarding the gut microbiota.[72] However, some researchers have questioned how informative mouse models are for research on human GI diseases.[73] Discrepancies in researching GI diseases, as well as whether these inferences are reliable, should be considered when translating the results from rodent models to humans.

Studies regarding taxonomic classification of the gut microbiota have found that approximately 85% of bacterial genera in the mouse gut microbiota are not present in the healthy human gut.[74] While the latest results show that the human gut microbiota is similar to that of both canines and mice, the canine gut microbiota is more similar to that of humans.[66,75] Rodent models remain the predominant animal models in gastrointestinal

microbiome research, and the research on mouse gastroenterology, neurology, immunology and genetics far surpasses that of other animal models.[76] Here, we discuss the similarities and biases in some representative cases related to GI diseases: obesity and IBD among humans, canines and mice.

In obesity:

Regarding the relevance of canine models in studying human obesity and its relation of canine models to mouse models, several factors should be considered. Obesity is usually accompanied by an altered gut microbiota.[77–79] Some common trends exist between obese humans and mice regardless of dietary intake. Gut microbiota diversity is lower in obese canines, and this lower diversity of the gut bacterial community is also observed in obese mice and humans.[46,79–83] Combined with obesity and diet, canines who consume both high-fat and high-protein diets have lower relative abundances of *Prevotella*, whereas the *Prevotella*-dominated enterotype in the human gut is associated with high carbohydrate consumption.[84] Additionally, all 3 species present an increased concentration of SCFAs.[15,85,86]

Increased bacterial Firmicutes/Bacteroidetes ratios have been shown in obese mouse gut microbiotas;[74,78] however, contradictory results showing decreased Firmicutes/Bacteroidetes ratios have also been reported.[85,87] In studies of the gut microbiota in obese humans, several conflicting reports also showed different Firmicutes/Bacteroidetes ratios, including a higher ratio,[82] lower ratio[85] and no difference.[88] However, the Firmicutes/Bacteroidetes ratio is higher in lean canines.[46] These conflicting results indicate that the Firmicutes/Bacteroidetes ratio in canines and mice should not be translated to obese humans, and further studies are needed.

Genome-wide association has shown that some genes associated with human obesity overlap with some identified obesity genes in mice.[89] Limited studies have investigated the genome-wide association in obesity between humans and canines, but recent research showed that canines share a significantly higher fraction of genes with humans than with mice;[66] therefore, obese canines may have more genes that overlap with those of obese humans.

In IBD:

Gut microbiome dysbiosis is usually observed with IBD and has been proposed as an inducing factor in IBD pathogenesis.[90,91] IBD has been widely reported in human patients, rodent models and canine models. Studies have shown that compared with the control group, the most significant phenomenon in IBD is microbiota dysbiosis in parallel with metabolic disorders in humans, mice and canines.[37,92–95] For example, *Enterobacteriaceae* are increased in all 3 species with IBD,[96–99] and *Enterobacteriaceae* acts in conjunction with the gut microbiota to cause IBD.[100] *Faecalibacterium prausnitzii* has anti-inflammatory effects, and research on human IBD has shown decreased *Faecalibacterium prausnitzii*.[101–103] *Faecalibacterium* spp. are also decreased in canine IBD,[39] while in mice with dextran sulfate sodium (DSS)-induced and 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis, *Faecalibacterium* spp. showed no significant changes.[104,105] The decrease in *Faecalibacterium prausnitzii* may cause a research bias in IBD because *Faecalibacterium prausnitzii* can secrete anti-inflammatory peptides and play an important role in IBD.[47,106] Another important bacterium, *Akkermansia*, is beneficial for gastrointestinal health, and *Akkermansia* is decreased in human IBD but increased in DSS-induced mouse models.[103,104,107] No published studies on canine IBD have discussed *Akkermansia*.

Metabolic disorders in IBD have been widely researched in human patients and mouse colitis models and have surpassed canine models. Fecal SCFAs are decreased by nearly the same amount in both humans and mice with IBD.[108–110] Canines with IBD have a lower diversity of several bacteria, which are thought to produce SCFAs.[41,111,112] Fecal SCFAs are also decreased in other canine GI diseases such as chronic enteropathy.[42] Serum metabolites differ in humans, mice and canines. Decreased serum alanine and valine have been observed in human IBD patients as well as mice, while both of these serum metabolites are increased in canines with IBD.[40,59,113,114] Alanine is an important transport metabolite for amino groups in animals; altered concentrations of alanine may indicate an altered amino acid turnover ability in IBD.[40] The difference in valine in IBD remains unknown. One possibility is the factor of different nutrient intakes among humans, mice and canines. However, no articles have been published to date regarding abnormal concentrations of serum metabolites in canine IBD. Deep analysis of the bacteria in both the canine and human gut provide opportunities for translating IBD research from canines to humans. A limitation of the studies on canine IBD is that few canines have been evaluated, and further research is essential to explain the similarities and biases of serum metabolites in humans and canines.

Future directions for canine gut microbiota research

While interesting similarities between the gut microbiotas in humans and canines have recently been addressed, comprehensive studies of these similarities and their mechanisms are unresolved. Research on the canine gut microbiota and GI diseases may predict results in humans. Symptoms of GI diseases in humans and canines have not been fully compared. Relationships between the canine gut microbiota and its role in the pathogenesis of canine GI diseases should be clarified. Culturomics has been widely used in studying the human gut microbiota for several years.[115–117] Culturomics involves isolating uncultured gut bacteria to the greatest extent possible under many culture conditions. Research on probiotic therapies for canine GI diseases and beneficial functions remains limited. Potential new probiotics must be isolated to treat canine GI diseases in compliance with ethical standards.

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