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Perspectives and Advances in Probiotics and the Gut Microbiome in Companion Animals

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33   **Abstract**

34

35   As the number of households that raise dogs and cats is increasing, there is growing interest in animal

36   health. The gut plays an important role in animal health. In particular, the microbiome in the gut is

37   known to affect both the absorption and metabolism of nutrients and the protective functions of the host.

38   Using probiotics on pets has beneficial effects, such as modulating the immune system, helping to

39   reduce stress, protecting against pathogenic bacteria and developing growth performance. The goals of

40   this review are to summarize the relationship between probiotics/the gut microbiome and animal health,

41   to feature technology used for identifying the diversity of microbiota composition of canine and feline

42   microbiota, and to discuss recent reports on probiotics in canines and felines and the safety issues

43   associated with probiotics and the gut microbiome in companion animals.

44   **Keywords:** Probiotics, Gut microbiome, Companion animal, Canine, Feline

45

4

Introduction

Terms such as 'companion animals' apply to households with pets, companion dogs and companion cats that are frequently encountered in the surroundings. The word 'companion', with which we are already familiar, refers to an animal that lives with humans and was first proposed by zoologist and Nobel Prize winner Konrad Lorenz at an international symposium held in Vienna, Austria in 1983 [1]. Households that raise these pets accounted for 29.7% of the total households in Korea, with 6.04 million households at the end of 2020 [2]. As the number of people raising companion animals is increasing, the relationship between humans and companion animals is further developing.

Most pet owners currently treat their pets as family, colleagues, and friends [3]. Pet humanization, a phenomenon that recognizes companion animals as family members and treats them as individuals with emotions, has been established as a global trend [4]. In Korea, the trend of pet humanization is also spreading, with 88.9% of companion households and 64.3% of general households agreeing to the phrase 'pets are part of the family' [2]. A typical example is that the pets do the same things as humans do, such as having birthday parties for dogs and cats, sleeping with the owner in the bed, and others. Companion animals have become an increasingly important part of human life, and therefore, the health and well-being of pets have increasingly attracted interest in recent decades [5].

Dogs and cats have evolved into carnivores with high-protein diets and have relatively simple gastrointestinal tracts (GITs) [5, 6]. Cats are carnivores that rely on high-protein animal tissues to meet their unique nutritional requirements in the wild and consume protein-containing feed to meet their nutrients in the case of household felines. They are metabolically adapted to low glucose utilization and high protein metabolism [5, 7]. Although dogs share many anatomical and metabolic characteristics with cats, they are metabolically omnivorous and can digest, absorb and metabolize significant amounts of carbohydrates [8].

The gut plays an important role in animal health, and the gastrointestinal tract contains a complex microbial community. A healthy gut is known to affect host physiology and well-being. This microbial ecosystem acts in several ways, affecting both the absorption and metabolism of nutrients and the protective functions of the host. Probiotics are defined as 'living microorganisms that provide health

5

73 benefits to the host when administered in appropriate amounts' [9]. Recently, gut-related probiotic

74 products aimed at pets, particularly dogs and cats, have also gained in popularity among owners [10].

75 The benefits of using probiotics for pets include their modulation of the immune system, help in

76 reducing stress, protection against infections caused by intestinal pathogens and growth performance

77 development [11]. As dogs and cats become family members, the number of studies about dogs and

78 cats has been increasing. Among their topics, knowledge about the gut microbiome and probiotics in

79 dogs and cats is still expanding. However, published papers on the application of probiotics in

80 companion animals are significantly limited compared to those in humans. The purpose of this review

81 is to describe the current knowledge about the gut microbial communities in dogs and cats in relation

82 to probiotics.

83

Probiotics for companion animals

Probiotics that are living and beneficial microbiota have been used in the nutrition of companion animals [9]. As people's desire to have their pets for a long time has increased, interest in probiotics has also attracted more attention [10]. Probiotics provide beneficial health effects to the host animal by altering the gastrointestinal flora. The gastrointestinal benefits for dogs and cats include maintaining a balanced and healthy gut microbiome, preventing diarrhea, and managing small intestinal bacterial overgrowth and inflammatory bowel disorders [12]. Since dogs and cats have different dietary needs and digestive systems, their needs for and effects from probiotics differ.

1) Probiotics for canines

Canines are considered animal models for the study of the human microbiome because of the high structural and functional similarity between the canine and human microbiomes [13]. The study of the dog microbiome can be predictive of the human microbiome. Thus, the study of dogs offers two advantages not only directly for dogs but also for its potentially benefits for humans [14]. Although the beneficial effects of probiotics have been extensively studied in humans and animals, the exact mechanisms of probiotic-based immune modulation are not entirely clear, and the efficacy of probiotic applications varies depending on many different factors [15]. Recent reports of using probiotics in canines are shown in Table 1 and Table 2.

Gastrointestinal (GI) disorders are one of the most common health problems in dogs [16, 17]. Regardless of the cause, most GI disorders present with acute or chronic diarrhea, or, in some cases, vomiting or anorexia [5, 18, 19]. Many previous studies have shown positive results regarding the treatment of dogs with different types of probiotics [20, 21]. Dogs on diets supplemented with 2×10^{10} CFU/day canine-derived probiotic *Bifidobacterium animalis* AHC7 had a significantly more rapid resolution of acute diarrhea than dogs that received placebo [22]. The administration of the probiotic potential of *Lactobacillus rhamnosus* MP01 and *Lactobacillus plantarum* MP02, two strains isolated from canine milk, decreased the *Faecalibacterium* in feces [23]. Supplementing with 5×10^9 CFU/day with the probiotic *Lactobacillus murinus* native strain (LbP2) in dogs improved their stool output, fecal

consistency, mental status, and appetite compared to the control [24]. A total of 15 adult female dogs who were given 2.3 x 10^8 CFU/day canine-origin probiotics (*Lactobacillus johnsonii* CPN23) exhibited increased fiber digestibility and concentrations of short-chain fatty acids in their feces and reduced fecal ammonia concentrations compared to the control [25]. Dogs that consumed 10^7-10^9 CFU/day canine-derived probiotic strain *Lactobacillus fermentum* CCM 7421 displayed an increased lactic acid bacteria population, reduced *Clostridia* population and some gram-negative bacterial genera. Additionally, dogs that consumed probiotics showed improved total protein, cholesterol and alanine transaminase in blood samples [26]. Three milliliters of 10^9 CFU/ml of the new potential probiotic *Lactobacillus fermentum* AD1 significantly increased total lipids and total protein and significantly decreased the glucose concentration in the bloodstream [27]. Dogs fed 1.04 x 10^9 CFU/ml *Bifidobacterium animalis* B/12 probiotic showed a significantly decreased concentration of triglycerides and albumin and increased acetic, acetoacetic, and valeric acid in feces [28]. Supplementing 10^8 CFU/ml canine-origin probiotic (*Lactobacillus johnsonii* CPN23) in adult female dogs decreased their plasma glucose and cholesterol levels and increased the high-density lipoprotein and low-density lipoprotein ratio [29]. Dogs receiving *Enterococcus faecium* DSM 32820 had optimal fecal consistency throughout the experiment, significantly stimulated phagocytic activity and a metabolic burst activity of leukocytes and lower serum glucose concentrations [30]. Heathy dogs receiving 5 x 10^9 CFU/kg *Lactobacillus acidophilus* D2/CSL showed higher body condition scores than the control dogs and there was a positive effect on their fecal consistency [31]. The probiotic feed additive contained three different bacterial strains, namely, *Lactobacillus casei* Zhang, *Lactobacillus plantarum* P-8, and *Bifidobacterium animalis* subsp. *lactis* V9, and promoted the average daily feed intake, improved average daily weight gain, increased beneficial bacteria and decreased potentially harmful bacteria [14]. The probiotic *Enterococcus faecium* SF68 improved diarrhea symptoms compared to the control, and *Giardia* cysts were eliminated [32]. Adding 5 x 10^8 CFU/day *Enterococcus faecium* SF68 significantly increased the triglyceride concentration and decreased the cholesterol concentration [33].

The gut microbiome greatly affects the health and disease of the host so maintaining it in good condition is important for the health of the host [34]. Many factors influence the composition of the gut

138 microbiome and aging is one of the greatest impacts [35]. After all, this aging which is defined as the

139 gradual changes that occur after maturation in various organs, resulting in decreased functional capacity

140 in the gut microbiome is thought to be somehow related to the health of the host [36]. Masuoka et al.

141 [34] conducted the experiment with dogs of 5 different age groups (pre-weanling, weanling, young,

142 aged and senile) and analyzed the composition of their intestinal microbiota of dogs in different age

143 groups. As a result, the composition of the dog's intestinal microbiota changed with age. *Lactobacillus*

144 and *Bifidobacterium* were found to decrease as the dog aged. This experiment showed that the gut

145 microbiome of dogs can be changed regarding the age at the level of bacterial groups and species.

146 Further studies are needed to be done to identify whether different probiotics are needed for different

147 phases of life.

148

149 **2) Probiotics for felines**

150 Cats have trillions of live bacteria in their bodies, which are mostly in their intestines [21]. Each cat's

151 bacterial population is different for individuals and can be changed based on diet, health status, and

152 lifestyle choices [37, 38]. During times of stress and infection, the microbiome balance can increase the

153 number of bad bacteria, disrupting the system's balance and potentially causing digestive problems such

154 as decreased appetite, vomiting, diarrhea or stool changes [39, 40]. Supplementing probiotics for felines

155 can be one of the best ways to add good bacteria to the cat body [21]. Recent reports of using probiotics

156 in felines are listed in Table 1 and Table 2.

157   Although many studies have investigated the use of probiotics in dogs, studies in cats are relatively

158 scarce. Few studies on probiotic usage in cats have been reported to date, and because of differences in

159 host physiologic characteristics and the diet, the probiotic efficacy in cats cannot be extrapolated from

160 studies in dogs [41]. The purpose of this review paper is to discuss various results about treating cats

161 with different types of probiotics. Kittens receiving 2.85-4.28 x 10^8 CFU/day *Enterococcus hirae*

162 showed high intestinal colonization and fecal shedding of live *Enterococcus hirae* during administration

163 [42]. Supplementing 2 x 10^8 CFU/day *Lactobacillus acidophilus* DSM13241 as a probiotic in healthy

164 adult cats increased the numbers of beneficial *Lactobacillus* and *L. acidophilus* groups in feces and

165 decreased the numbers of *Clostridium spp.* and *Enterococcus faecalis.* It also decreased the fecal pH

166 and plasma endotoxin concentrations and resulted in systemic and immunomodulatory changes in

167 treated cats [41]. Kittens fed 5 x 10^9 CFU/day *Enterococcus faecium* strain SF68 showed a significantly

168 higher percentage of CD4+ lymphocytes than controls [43]. Healthy adult cats fed 5 x 10^9 CFU/kg

169 *Lactobacillus acidophilus* D2/CSL had better results in terms of their fecal quality parameters and had

170 increased *Lactobacillus* counts and decreased total coliform bacteria counts [44]. The percentage of cats

171 with diarrhea was significantly lower in the 2.1 x 10^9 CFU/day *Enterococcus faecium* SF68 group than

172 in the control group [45]. Young adult cats receiving *Enterococcus faecium* SF68 had significantly

173 lower total diarrhea scores for days 1-11 compared to the control. Additionally, feeding *Enterococcus*

174 *faecium* SF68 could lessen some associated clinical abnormalities [46]. Cats with chronic feline

175 herpesvirus-1 (FHV-1) infection who were fed *Enterococcus faecium* SF68 showed fecal microbial

176 diversity throughout the study, indicating a more stable microbiome. The treatment also lessened the

177 morbidity associated with chronic FHV-1 infections [47]. Healthy cats with 5 x 10^9 CFU from a mixture

178 of seven bacterial species per day (Proviable® -DC) showed an increased abundance of probiotic

179 bacteria in the feces. Probiotics also improved diarrhea after 21 days of feeding [48]. Cats with chronic

180 gingivostomatitis that were fed 1 x 10^8 CFU/ml *Lactobacillus plantarum* showed many positive results

181 in gingivostomatitis symptoms. There was an improvement in the time of recurrence, and the symptoms

182 of chronic feline gingivostomatitis disappeared after two weeks of administration. Additionally,

183 ulceration, inflammation and oral cavity pain decreased, and thalism and halitosis disappeared [49].

184 Giving multistrain probiotic products to 8-month-old male cats with feline idiopathic cystitis effectively

185 managed this disease due to the effects of bactericidal, anti-inflammatory, and immunomodulatory

186 actions [50].

187   The health and disease of the host are affected by gut microbiota, maintaining the gut microbiota is

188 getting more important as cats get aged. Masuoka et al. [51] conducted the experiment with cats of 5

189 different age groups (pre-weanling, weanling, young, aged and senile) and analyzed the composition of

190 their intestinal microbiota of cats in different age groups. The results suggested that the composition of

191 the cat's gut microbiome changed with age, whereas the change was different from that of dogs.

192   *Bifidobacterium* which predominated in the gut of dogs did not appear to be important in the gut of cats.

193   Instead, *enterococcus* appeared to be the main lactic acid-producing bacteria in cats. Ultimately, the

194   results of this study indicated that the compositions of the gut microbiome between dogs and cats are

195   different and those compositions are changing with aging. Not only are different probiotics might need

196   for dogs and cats but also for regarding aging. Further studies are needed to use different probiotics for

197   different phases of life.

198

199

200   **Gut microbiome for companion animals**

201   **1) Gut microbiome and nutrient metabolism**

202   Microorganisms affect the absorption of nutrients in the host and provide beneficial metabolites in

203   return for using host nutrients [52]. Each intestine harbors a different unique microbial ecosystem

204   due to anatomical and physiological differences [53]. Additionally, each animal harbors a different

205   and unique microbial profile. For example, at the species and strain levels, only a few overlap between

206   individual animals. However, the bacterial phyla, order and genera are shared by most mammals [54].

207   The most predominant bacterial gene category in the canine gut is carbohydrate metabolism, such as

208   that related to mannose, oligosaccharide and raffinose metabolism. The fermentation of carbohydrates

209   by colonic organisms such as *Bacteroides*, *Roseburia*, *Ruminococcus* and *Lachnospiraceae* results in

210   the synthesis of short-chain fatty acids (SCFAs), such as butyrate, propionate and acetate, which are

211   sources of energy for the host [20]. SCFAs have beneficial effects on host health, including

212   immunomodulatory effects, anti-diarrheic effects, and a regulatory effect on GI motility. In the case of

213   felines, which are obligate carnivores, consuming raw meat increased *Clostridium* and *Eubacterium*,

214   which are known to produce SCFAs [55].

215   The synthesis of vitamin K and several components of vitamin B are important functions of the

216   intestinal microbiota [56, 57]. Vitamin K, which is included in fat-soluble vitamins, plays an important

217   role in prothrombin coagulation factor activity. Therefore, there is a risk of intestinal bleeding in cases

218   of vitamin K deficiency [58]. Vitamin B12 (also known as cobalamin) is important for many aspects of

11

219 a dog's health [59]. It is crucial for a healthy nervous system and brain function as well as for the

220 formation and growth of blood cells [60]. Additionally, it is needed to maintain healthy digestion [61].

221 As a result of a metagenome analysis using dog feces, genes affecting lipoprotein lipase activity in

222 adipocytes were identified in intestinal microbial genes, confirming that microorganisms are also

223 related to lipid metabolism [62].

224

225 **2) Gut microbiome and the immune system**

226 The microbiome plays an important role in the immune system of the intestinal tract. In particular, early

227 microbial exposure significantly affects gut microbiome formation and immune modulation, which

228 affects susceptibility to intestinal diseases [63]. When comparing animals born through vaginal delivery

229 with germ-free animals through cesarean section, the germ-free animals have fewer and smaller Peyer's

230 patches, mesenteric lymph nodes and CD4+ T cells in the lamina propria of the gut wall [64]. In germ-

231 free animals, a reduction in B cells, macrophages and neutrophils was confirmed [65]. Additionally, in

232 germ-free animals, immunoglobulin was found at a level of 2%, which was significantly lower than that

233 in normal healthy animals [66]. The microbiome also plays a role as a signal indicating health [65].

234 This characteristic is expected because animals evolved in coexistence with symbiotic microorganisms

235 for a very long time [67]. Microorganisms that coexist with animals communicate directly and

236 effectively with their host's immune system through metabolites and nutrients [64, 65].

237

238 **3) Identifying diversity in the canine and feline microbiomes**

239 The intestine is a major part of the body that influences host health. Numerous microbes form the

240 complex microbial community in the gastrointestinal tract (GIT). Disrupting the gut microbiome may

241 cause dysbiosis and lead to several diseases and disorders, such as diarrhea, allergies and obesity [21].

242 Gastrointestinal disease caused by the dysbiosis of the gut microbial community is also generally

243 observed in dogs and cats [68, 69]. Knowing the diversity and taxonomic bacterial distribution of the

244 gut microbiota of healthy dogs and cats is important as a baseline in future studies evaluating

245 gastrointestinal diseases in dogs and cats [70, 71].

246   Previous studies have focused on the cultivation of intestinal content to characterize and identify the

247   microbiota [72-74]. Most of the bacterial groups cultivated from the canine intestine belonged to

248   *Enterobacteriaceae, Bacteroides*, *Clostridium*, *Lactobacillus*, and *Bifidobacterium* spp. [75, 76].

249   However, culturing the bacteria to evaluate the complex diversity of the microbiota had limitations.

250   Bacterial species that can be cultivated by using bacterial culture techniques are only a small portion of

251   microbiota composition [77, 78], anaerobic bacteria can be easily damaged during sample handling [79,

252   80], the cost used for culture techniques is expensive [80], a great amount of time is used for isolation

253   and cultivation [80, 81]. A novel molecular method that uses the 16S ribosomal RNA (rRNA)-enabled

254   the evaluation of the diversity and abundance of bacteria in the sample without culturing [82, 83].

255   The development of next-generation sequencing technologies has helped to characterize bacterial

256   communities and to understand interactions between hosts and bacteria. Using next-generation

257   sequencing, dog and cat organ microbiotas have been described. These microbiota include those of the

258   gastrointestinal tract [70, 84-86], skin [87], oral cavity [88] [89], nasal cavity [90], and vagina [91].

259

260   **4) Composition of canine and feline microbiome**

261   All animals, including dogs and cats, harbor numerous microorganisms in the GIT [8]. Dogs and cats

262   have different microbiota compositions and they also differ in the same species [21]. There are lots of

263   factors that can affect microbiota compositions such as age [92-94], breed [8, 95, 96], diet composition

264   [39, 92, 94], disease [92, 93], environment [92, 96, 97], food type [93, 98] and sex [99, 100].

265   The gut microbiome which is highly related to a healthy life could be affected by dogs' breed. There

266   was a relationship between gastrointestinal conditions and dog breeds [101, 102]. According to You

267   and Kim's experiment [103], there was a difference in microbial composition in Poodle and Maltese

268   groups. Also, phylum Fusobacterium was differed by breeds (Maltese, Poodle, and Miniature

269   Schnauzer). From these data, they suggested that there might be differences in the gut microbiome

270   composition depending on the dog breeds. According to Lehtimäki's experiment [104], living

271   conditions have a significant impact on the skin microbiome in humans and dogs, but not the gut

272   microbiome. Dogs living in rural and urban environments participated in the study. The skin

microbiome was more diverse among individuals in rural areas compared to urban areas. This study showed that the living environment had a much greater effect on the skin microbiome than the guts of dogs. Experiments on changes in the gut microbiome of cats according to breeds and living environments have been limited. Further research is needed as with dogs. According to Older's experiment [96], breed and living environment played an important role in shaping the cat skin microbiome. In particular, it seems that the hair coat and grooming according to the cat breeds have a great influence on the microbiome of the cat's skin microbiome.

The bacterial count in the stomach is between 10^4 and 10^5 CFU/g [105]. In the duodenum and jejunum, the bacterial counts are generally low (10^5 CFU/g) but can reach 10^9 CFU/mL in some dogs and cats [106]. The ileum contains an increasing number of diverse microbiota, mostly at 10^7 CFU/mL. The bacterial counts in the colon are between 10^9 and 10^{11} CFU/g [38, 73].

The healthy canine stomach has a comparably low number of total bacteria. Most belonged to Proteobacteria (99.6%), and few belonged to Firmicutes (0.3%). The dominant species are *Helicobacter* and *Lactobacillus* spp. [85]. Using 16S rRNA sequences, four phyla (Firmicutes, Fusobacteria, Bacteroidetes and Proteobacteria) predominated in the small intestine [70]. The duodenum of healthy canines consisted of six phyla. Firmicutes predominated followed by Proteobacteria, Bacteroidetes, Spirochaetes, Fusobacteria and Actinobacteria [37, 107]. Healthy dog microbiota in the jejunum were evaluated, and the most predominant phylum was Proteobacteria (46%), followed by Firmicutes (15%), Actinobacteria (11.2%), Spirochaetes (14.2%), Bacteroidetes (6.2%) and Fusobacteria (5.4%) [84]. The ileum microbiota of healthy dogs predominantly consists of Fusobacteria, Firmicutes and Bacteroidetes [70].

Lactobacillales was present in all parts of the intestines (22% in the duodenum and 10% in the jejunum). Enterobacterales were more frequently detected in the small intestine than in the colon. Clostridiales were highly abundant in the duodenum (40%), jejunum (39%), ileum (25%) and colon (26%) [70]. Facultative anaerobic *Lactobacillus* strains predominated in the jejunal microbiota, and *L. acidophilus* was the most abundant among them [108]. In the jejunal samples, facultative anaerobic and anaerobic bacteria were similarly detected, while anaerobic bacteria predominated in the fecal samples.

The number of bacteria in the jejunum was 10^2 to 10^6 CFU/g, while the number in the feces was 10^8 to 10^{11} CFU/g. Despite the lower number in the small intestine, some microbial groups were more prevalent in the small intestine than in feces: staphylococci, 64% versus 36%; non fermentative gram-negative rods, 27% versus 9%; and yeasts, 27% versus 5%[73].

Firmicutes, Bacteroidetes, Fusobacteria, Proteobacteria and Actinobacteria were the most abundant phyla in the fecal microbiota of healthy dogs [70, 86, 109]. However, Fusobacteria (39.17%) were dominant, followed by Bacteroidetes (33.36%) and Firmicutes (15.81%) in healthy adult Miniature Schnauzer dogs [86], while the abundances of Fusobacteria, Bacteroidetes and Firmicutes were similar (approximately 30% each) in six Hound dogs [109].

Clostridia was the most predominant bacterial class in the dog fecal microbiota [109]. At the genus level, *Lactobacillus* was the most predominant, followed by *Bifidobacterium*, *Enterococcus*, *Streptococcus* and *Pediococcus,* in the dog fecal microbiota [110]. Many *Lactobacillus* spp. including *L. casei*, *L. salivarius*, *L. rhamnosus*, *L. mucosae*, *L. fermentum*, *L. reuteri*, *L. animalis*, *L. acidophilus* and *L. johnsonii* were the most frequently isolated ones from the feces. *L. reuteri, L. animalis*, and *L. johnsonii* were the most predominant species in dogs [110-112]. *Weissella confuse*, *Pediococcus acidilactici*, *Enterococcus* spp. and *Bifidobacterium animalis* ssp. lactis were also frequently isolated from dog feces [111-113]. At the fungal kingdom-phylum level, Ascomycota, Basidiomycota, Glomeromycota**,** and Zygomycota were detected in dog feces [109].

In the skin microbiota, the most predominant phyla and families were Proteobacteria and *Oxalobacteriaceae* [87]. At the oral microbiota phylum level, Bacteroidetes (60%) was the most predominant, followed by Proteobacteria (20.8%), Firmicutes (11.4%), Fusobacteria (4.7%) and Spirochaetes (1.7%). At the genus level, the oral microbiota consisted of *Porphyromonas* (39.2%), *Fusobacterium* (4.5%), *Capnocytophaga* (3.8%), *Derxia* (3.7%), *Moraxella* (3.3%) and *Bergeyella* (2.7%) [88]. In the nasal microbiota of healthy dogs, *Moraxella* spp. was the most abundant species, followed by *Phyllobacterium* spp., *Staphylococcus* spp., and *Cardiobacteriaceae* [90]. The most frequently isolated bacteria from the dog's vaginal tract were *Lactobacillus*, *Escherichia coli* and *Staphylococcus pseudointermedius* [21, 91].

The feline gastrointestinal tract has different bacterial species than other animals. *Helicobacter* is known to reside in the stomachs of cats [114]. For the microbiota composition of the GI (stomach, duodenum, jejunum, ileum, and colon) contents, which were collected from 5 healthy felines, Firmicutes (68%) predominated, followed by Proteobacteria (14%), Bacteroidetes (10%), Fusobacteria (5%) and Actinobacteria (4%). At the order level, Clostridiales (54%) prevailed, followed by Lactobacillales, Bacteroidales, Campylobacterales, and Fusobacteriales [37]. Based on several studies, it is known that *Bacteroides* spp., *Clostridium* spp., *Enterococcus* spp., *Streptococcus* spp., *Fusobacteria* spp., and *Eubacteria* spp. are present in the small intestines of felines [69, 115]. Representative lactic acid bacteria present in the gastrointestinal tract of felines include *L. acidophilus*, *L. salivarius, L. johnsonii*, *L. reuteri* and *L. sakei*, which are typical intestinal lactic acid bacteria found in animals, including humans, although the amount varies by individual [21, 37]. The major phyla in the feline fecal microbiota were Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria [69, 109, 116]. These four phyla make up more than 99% of the fecal microbiota [69]. Handl [109] reported that Firmicutes was the most prevalent phylum in the fecal microbiota, followed by Bacteroidetes and Actinobacteria; however, Tun [116] reported that Bacteroidetes was the most predominant phylum, followed by Firmicutes and Proteobacteria. *Bacteroides*, *Fusobacterium*, and *Prevotella* were the most predominant genera in the feline fecal microbiota, which indicated that these genera play a major role in the feline intestine [117]. Fungi, archaea and viruses compose a minor part of intestinal microbial communities. *Ascomycota* was the only phylum of fungi detected in cats [109, 116].

Malassezia spp. were the most prevalent fungi in feline skin mycobiota. *M. restricta* and *M. globosa* were the most predominant fungal species in all cat breeds [96]. *M. pachydermatis* is known as a yeast that is present in the skin microbiome, yet it can also act as a pathogen that can cause dermatitis [118]. The phylum level of the oral microbiota is generally conserved between cats. These phyla are predominated by Proteobacteria (75.2%), followed by Bacteroidetes (9.3%), Firmicutes (6.7%), SR1 (2.7%), Spirochaetes (1.8%), Fusobacteria (1.3%), and Actinobacteria (0.6%) [89]. The composition of the canine and feline microbiota is shown in Figs. 1 and 2.

354

Safety issues of probiotics and the gut microbiome in companion animals

The Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO) defined probiotics as "live microorganisms, which when administered in adequate amounts, confer a health benefit on the host" [9]. Tremendous scientific evidence for the efficacy of probiotic candidates has been available for decades, but insufficient information about their safety is available. While known to be safe in general, a few adverse effects associated with probiotic use have been documented in patients [119, 120]. Moreover, there is a lack of information on the inherent characteristics of each probiotic strain that may be associated with health risks [121].

The European Food Safety Authority (EFSA) recommended the qualified presumption of safety (QPS) status for microorganisms used in feed and food production in 2003. Based on the QPS guidelines, microbes that produce toxins or possess virulence factors that may contribute to their pathogenicity cannot be used as probiotics. In addition, it must be ensured that there are no acquired genes encoding antimicrobial resistance (AMR). The existence of knowledge, including a history of use, ecology, industrial application, clinical reports, and a public database, is considered important evidence for evaluating the safety of microbial species [122]. QPS list includes several taxonomic units for bacteria, yeasts, and viruses [123], of which *Lactobacillus* and *Bifidobacterium* are representative because their reasonable certainty of no harm has been supported by an extensive record of safe use [124].

With the recent focus on the beneficial effects of probiotics in companion animals and their relationship to gut microflora and health, probiotic products are being increasingly marketed in the form of feed additives, dietary supplements, and probiotic-containing foods [125]. Although there have been no reports of adverse events when probiotics are administered to small animals, safety concerns remain to be addressed [126]. The microorganisms used in feed additives require safety verification for target animals, manufacturers, and owners/consumers. In particular, *Enterococcus*, known as canine and feline intestinal commensal bacteria, have been used as probiotics for small animals, but their use is restricted in some countries because of the risk of host infection by AMR gene transfer [125, 127]. Rinkinen [128] demonstrated that some *Enterococcus faecium* strains promoted the adherence of the zoonotic pathogen

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381 *Campylobacter jejuni* in the intestines of canines. Notably, the administration of *E. faecium* strain SF68

382 (deposited as strain NCIMB 10415), which originated from infant feces in 1968, reduced the occurrence

383 of diarrhea in dogs and cats housed in animal shelters [45], and it has been verified that the strain may

384 not cause any safety concerns for companion animals and their owners [129-131]. Due to these

385 conflicting outcomes, stringent safety evaluations are required in a strain-specific manner with regard

386 to probiotic use.

387   Host specificity is considered an important criterion for selecting probiotic candidates, primarily due

388 to differences in physiological structure, immune systems, and microbial composition [132, 133].

389 However, most commercial probiotics used as feed additives originate from humans and are verified by

390 human-based methods and criteria [134]. The clinical results from Weese and Anderson [135] showed

391 that *Lactobacillus rhamnosus* strain GG, a commercial probiotic strain isolated from a healthy human

392 gastrointestinal tract, may not be suitable for use in canines because of its short persistence. In addition,

393 in an in vitro test, probiotic strains of canine origin inhibited the adhesion of enterotoxigenic

394 *Clostridium perfringens* to canine jejunal chyme more efficiently than non-canine strains [128]. Recent

395 studies have focused on strains isolated from the intestines of healthy dogs and cats to demonstrate their

396 impact on pathogen inhibition, attenuation of inflammatory status, and modulation of the gut

397 microbiome [136, 137]. Host specificity has been discussed with respect to probiotic efficacy in most

398 publications but must also be addressed from a safety perspective. Furthermore, clinical outcomes for

399 the safe use of probiotics in target animal species should be documented. Given the host specificity and

400 broad diversity of potential probiotic candidates for small animals, the availability of novel species with

401 no record of use requires attention [133]. For a complete characterization, documentation of genetic and

402 biochemical properties and long-term clinical trials are needed. Additional efforts are required to

403 standardize safety assessment methods for novel species to be considered QPS or as having a generally

404 recognized as safe status based on the opinions of regulatory bodies and expert panels [138].

405   Quality control issues associated with probiotics relate to products intended for animals as well as

406 humans [21]. The global market for probiotics for companion animals is growing, but insufficient

407 quality control regulations create serious problems for the safety of consumers and target animals. Some

408 investigators have disclosed that many commercial animal probiotics or pet foods that claim to contain

409 probiotics did not contain microbial species listed on the label or even contain other species. Moreover,

410 bacterial viability, a key concept to stipulate probiotics, was inconsistent with labeled values expressed

411 in colony-forming units [139-141]. The current low level of quality control may lead to exposure to

412 unknown health risks not only for the animals but also for the owners and the environment. Recent

413 advances in meta-omics technologies (metagenomics, metatranscriptomics, metaproteomics, and

414 metabolomics) have promoted a correct evaluation of the quality of probiotic products. For the

415 multistrain product VSL#3, Mora and colleagues [142] successfully identified microbial taxa with

416 metagenomics and viability by flow cytometry and confirmed reproducibility using metaproteomics.

417 Metagenomic approaches have also enabled the analysis of genes related to safety concerns in a culture-

418 independent manner. Stringent oversight by regulatory bodies, high manufacturer awareness, and the

419 development of rigorous evaluation methods by researchers are needed for the safe selection and

420 production of probiotic candidates intended for both companion animal and human use.

421

422

423 **Conclusion**

424 Although not enough research has been conducted on the probiotics and gut microbiome thus far,

425 because the number of companion animal people and the companion animal market are growing,

426 research related to the companion animal microbiome is also growing. Recently, in-depth research has

427 been performed to identify the functionality of probiotics and the gut microbiome of companion animals

428 and to make them with various materials, ranging from feed, snacks, supplies, and treatments for the

429 diseases of companion animals. The current evidence suggests that specific probiotic strains and/or their

430 defined combinations may be useful in canine and feline nutrition, therapy and care. However,

431 probiotics and the gut microbiota used in the present study are of human origin; thus, the companion

432 animal-specific health benefits are not unclear. Therefore, the most important step is to secure pet-

433 originated microorganisms for their health claims. Moreover, detailed *in vivo* designs and trials using

434 companion animals are needed to identify and characterize newly isolated pet-originated microbiomes

435   with an impact on health maintenance in both dogs and cats. Corroborations of these health-promoting

436   effects and microbiological safety issues should be assessed regarding potential probiotics and the gut

437   microbiome for animal health and welfare.

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440   **Acknowledgments**

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Table 1. List of canine and feline origin bacterial strains used as probiotic properties in canines and felines

Bacterial strains	Amount	Source	Group	Tested for	Result	Reference
Bifidobacterium animalis AHC7	2×10^{10} CFU/day	Canine	Young adult dogs with acute diarrhea	Assessment for managing acute diarrhea	Reduced diarrhea compared to placebo group	[22]
Lactobacillus rhamnosus MP01 *Lactobacillus plantarum* MP02	10^9 CFU/day	Canine	1 month old puppies	Assessments for preventing gastrointestinal infection in puppies	Significantly increased *Lactobacillus* and *Faecalibacterium* in fecal Significantly increased SCFAs concentration in feces Prevented gastrointestinal infection	[23]
Lactobacillus murinus LbP2	5×10^9 CFU/day	Canine	Dogs with canine distemper virus (CDV)-associated diarrhea	Assessment of fecal and mental status	Fecal consistency, mental status and appetite were significantly improved	[24]
Lactobacillus johnsonii CPN23	2.3×10^8 CFU/day	Canine	Adult female Labrador dogs	Assessment of nutrient digestibility and fecal fermentative metabolites	Increased crude fiber digestibility Increased concentrations of SCFAs in feces Reduction in fecal ammonia concentration	[25]
Lactobacillus fermentum CCM 7421	10^7- 10^9 CFU/day	Canine	Dogs suffering from gastrointestinal disorder	Assessment of blood samples and composition of the fecal microbiome	Improved total protein, cholesterol and ALT in blood samples Increased lactic acid bacteria population and decreased clostridia population along with some of the gram-negative bacterial genera Modulate liquid feces to normal consistency (dogs with diarrhea)	[26]
Lactobacillus fermentum AD1	3 ml of 10^9 CFU/ml	Canine	Healthy dogs	Assessment of blood sample and composition of fecal microbiome	Significantly increased total lipid and total protein in the blood Significantly decreased glucose concentration in the bloodstream Significantly increased the number of lactobacilli and enterococci in the feces	[27]

Bifidobacterium animalis B/12	1 ml of 1.04 x 10^9 CFU/ml	Canine	Healthy dogs	Assessment of blood samples and composition of fecal microbiome	Significantly decreased concentration of triglycerides and albumin in blood serum Increased ALT and ALP Increased acetic, acetoacetic and valeric acids in feces	[28]
Lactobacillus johnsonii CPN23	10^8 CFU/ml (0.1 ml/kg BW)	Canine	Adult female dogs	Assessment of blood sample profile	Decreased plasma glucose and cholesterol level Increased HDL/LDL ratio	[29]
Enterococcus faecium DSM 32820	10^9 CFU/day	Canine	Healthy dog	Assessment of blood sample profile	Decreased serum glucose concentration	[30]
Lactobacillus acidophilus DSM13241	2×10^8 CFU/day	Feline	Healthy adult cats	Assessment for improving intestinal health in cats	Increased numbers of beneficial *Lactobacillus* and *L. acidophilus* groups in feces and decreased numbers of *Clostridium* spp. and *Enterococcus faecalis* Decreased fecal pH and plasma endotoxin concentrations resulting in systemic and immunomodulatory changes in treated cats	[41]
Enterococcus hirae	$2.85\text{-}4.28 \times 10^8$ CFU/day	Feline	Kittens	Assessment for preventing atypical Enteropathogenic *E. coli* (EPEC) in kittens	Highly effective at promoting intestinal colonization and fecal shedding of live *E. hirae* during administration. Ameliorated the effects of atypical EPEC experimental infection on intestinal function and water loss	[42]
Enterococcus faecium strain SF68	5×10^9 CFU/day	Feline	Kittens	Effects of *Enterococcus faecium* strain SF68 supplementation on immune function	The percentage of CD4+ lymphocytes was significantly higher in the treatment group	[43]

Table 2. List of bacterial strains of non-canine and non-feline origin when used for their probiotic properties in canines and felines

Bacterial strains	Amount	Source	Group	Tested for	Result	Reference
Lactobacillus casei Zhang *Lactobacillus plantarum* P-8 *Bifidobacterium animalis* subsp. Lactis V9	2×10^9 CFU/g (2 g for young, 4 g for training, 10 g for elderly dogs)	*Lactobacillus casei* Zhang (koumiss) *Lactobacillus plantarum* P-8 (Fermented dairy products in China) *Bifidobacterium animalis* subsp. Lactis V9 (Feces of a healthy Mongolian child)	Young, training and elderly dogs	Assessment of nutrition, immunity and composition of fecal microbiome	Promoted the average daily feed intake of elderly dogs Improved average daily weight gain om all dogs Enhanced the level of serum IgG, IFN-α, and fecal secretory IgA (sIgA), while reducing the TNF-α. Increased beneficial bacteria and decreased potentially harmful bacteria	[14]
Lactobacillus acidophilus D2/CSL	5.0×10^9 CFU/kg of diet	GI tract of a healthy adult chicken	Healthy dogs	Assessment of nutritional and fecal status	Higher body condition score than control group Positive effect on fecal consistency	[31]
Enterococcus faecium SF68	5×10^8 CFU/day	Feces of a healthy breast-fed baby	Dogs with diarrhea	Assessment of the effect of administering metronidazole with *Enterococcus faecium* SF68 to treat diarrhea	Dual therapy that administrates metronidazole with *Enterococcus faecium* SF68 improved diarrhea more than administering metronidazole alone *Giardia* cysts were eliminated from the dual treatment group	[32]
Enterococcus faecium SF68	5×10^8 CFU/day	Feces of a healthy breast-fed baby	Healthy dogs	Assessment of blood sample profile	Mean cholesterol concentration significantly decreased Mean triglyceride concentration significantly increased	[33]
Lactobacillus acidophilus D2/CSL (CECT 4529)	5×10^9 CFU/kg of food	Conventional foods such as milk, yogurt and dietary supplements	Healthy adult cats	Assessment of the effects on nutritional condition and fecal quality	Improved fecal quality parameters Increased *Lactobacillus* count and decreased total coliform bacteria counts	[44]

Enterococcus faecium SF68	2.1 x 10^9 CFU/day	A healthy breast-fed newborn baby	Cats	Effects on *Enterococcus faecium* SF68 in diarrhea	The percentage of cats with diarrhea was significantly lower in the probiotic group when compared with the placebo group	[45]
Enterococcus faecium SF68	1/4 can of canned food mixed with *Enterococcus faecium*	A healthy breast-fed newborn baby	Young adult cats	Description of the gastrointestinal abnormalities associated with the administration of amoxicillin-clavulanate to cats and an assessment of whether feeding with *Enterococcus faecium* SF68 could ameliorate those abnormalities	The total diarrhea scores for days 1-11 were significantly lower in the cats fed *Enterococcus faecium* SF68 compared to the cats fed the placebo Feeding *Enterococcus faecium* SF68 can lessen some associated clinical abnormalities	[46]
Enterococcus faecium SF68	5 x 10^8 CFU/day	A healthy breast-fed newborn baby	Cats with chronic Feline Herpes virus-1 (FHV-1) infection	Assessment of the effect of feeding *Enterococcus faecium SF68* in clinical signs of FHV-1 infection	Fecal microbial diversity was maintained throughout the study in cats supplemented with *Enterococcus faecium* SF68, indicating a more stable microbiome in cats receiving *Enterococcus faecium* SF68 Lessened morbidity associated with chronic FHV-1 infection in some cats	[47]
Proviable®-DC (7 bacterial species)	5 x 10^9 CFU of a mixture of seven bacterial species per day	Multistrain probiotic product	Adult cat	Improvement in stool character	Improved diarrhea symptoms after 21-day feeding	[48]
Lactobacillus plantarum	1 × 10^8 CFU/mL	Mare's milk	Cats with chronic gingivostomatitis	Assessment of preventive and therapeutic oral pathology	The administration of the probiotic to the two immunosuppressed cats affected by gingivostomatitis led to an improvement in the time of recurrence The symptoms of chronic feline gingivostomatitis disappeared after two weeks of administration The ulceration, inflammation and pain of the oral cavity decreased, thalism and halitosis disappeared	[49]

Probiotic combination	The probiotic with *Lactobacillus casei* 4 x 10^8 CFU, *Lactobacillus rhamnosus* 3 x 10^8 CFU, *Lactobacillus acidophilus* 5 x 10^7 CFU, *Lactobacillus bulgaricus* 1 x 10^7 CFU, *Bifidobacterium infantis* 4 x 10^7 CFU, *Bifidobacterium breve* 5 x 10^7 CFU, and *Streptococcus thermophilus* 1 x 10^8 CFU	Multistrain probiotic product	8-month-old male cats	Management of feline idiopathic cystitis (FIC) using probiotics	Probiotic combination treatment effectively managed this disease due the effect of bactericidal, anti-inflammatory, and immunomodulatory actions	[50]
Proviable®-DC (7 bacterial species)	5 x 10^9 CFU of a mixture of seven bacterial species per day	Multistrain probiotic product	Healthy cats	Assessment of a multispecies on the fecal microbiome of healthy cats	Increased abundance of probiotic bacteria in the feces of healthy cats	[85]

Figure legends

Stomach
10^4 to 10^5 CFU/g
(Phylum)
Firmicutes
Proteobacteria
(Genus)
Lactobacillus
Helicobacter

Small intestine
10^5 to 10^9 CFU/g
(Phylum)
Firmicutes
Proteobacteria
Bacteroidetes
Spirochaetes
Fusobacteria
Actinobacteria
(Genus)
Lactobacillus
Staphylococcus

Nasal
(Genus)
Staphylococcus
Moraxella
Phyllobacterium

Oral
(Phylum)
Firmicutes
Proteobacteria
Bacteroidetes
Spirochaetes
Fusobacteria
(Genus)
Fusobacterium
Porphyromonas
Capnocytophaga
Derxia
Moraxella
Bergeyella

Skin
(Phylum)
Proteobacteria

Large intestine
10^9 to 10^{11} CFU/g
(Phylum)
Firmicutes
Proteobacteria
Bacteroidetes
Fusobacteria
Actinobacteria
(Genus)
Lactobacillus
Staphylococcus
Streptococcus
Pediococcus
Enterococcus
Bifidobacterium
Weissella

Urogenital tract
(Genus)
Lactobacillus
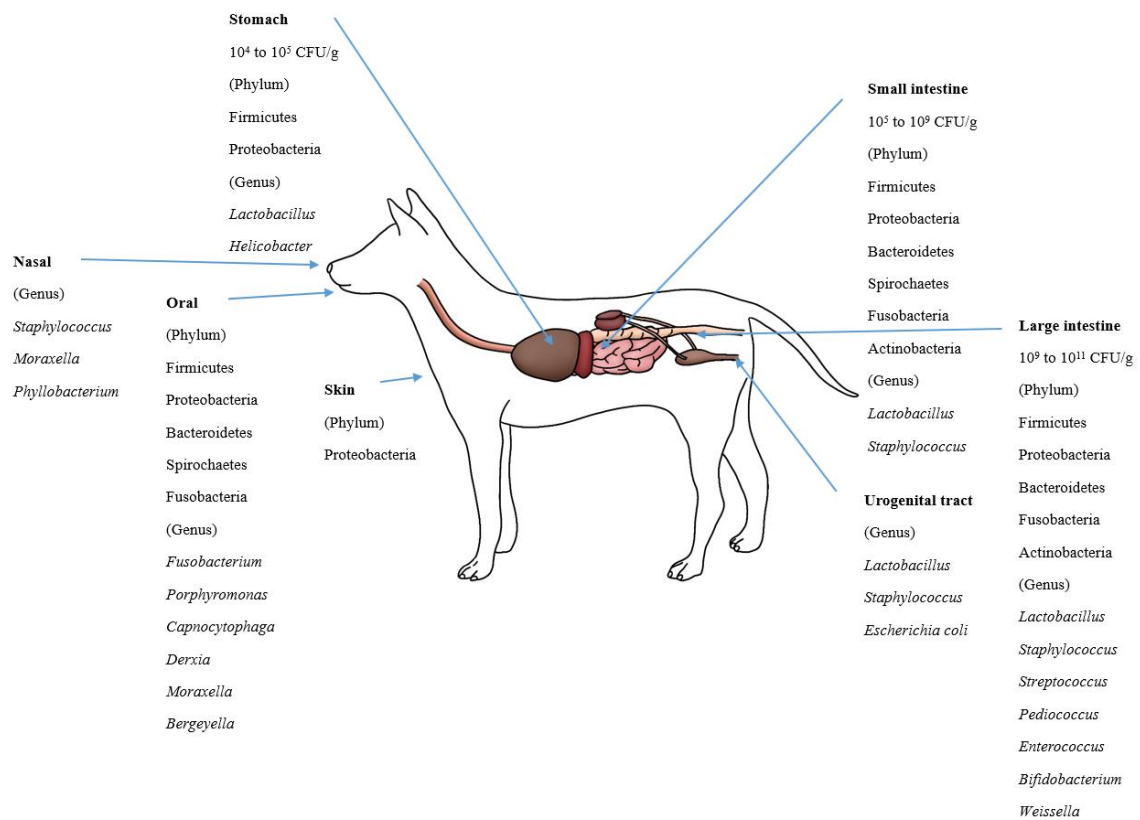*Staphylococcus*
Escherichia coli

Figure 1. The dynamic community of nasal, oral and gut microbiota in canines (adapted from [21], [70], [86], [87], [88], [90], [91], [73], [38], [107], [37], [108], [109], [110], [111], [112], and [113])
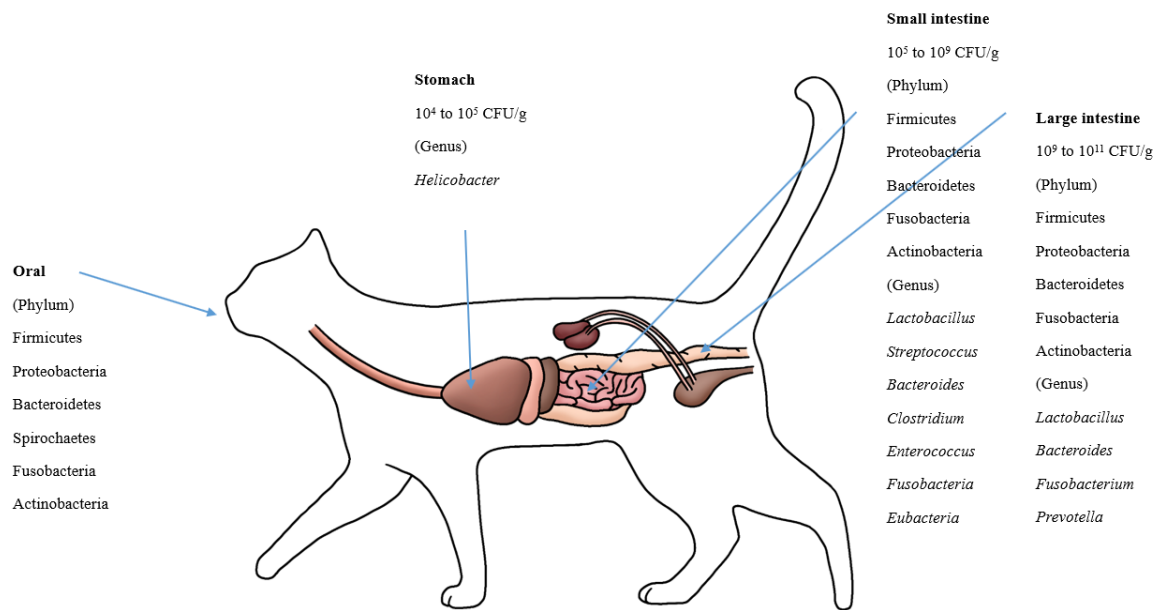
Stomach

10^4 to 10^5 CFU/g

(Genus)

Helicobacter

Small intestine

10^5 to 10^9 CFU/g

(Phylum)

Firmicutes

Proteobacteria

Bacteroidetes

Fusobacteria

Actinobacteria

(Genus)

Lactobacillus

Streptococcus

Bacteroides

Clostridium

Enterococcus

Fusobacteria

Eubacteria

Large intestine

10^9 to 10^{11} CFU/g

(Phylum)

Firmicutes

Proteobacteria

Bacteroidetes

Fusobacteria

Actinobacteria

(Genus)

Lactobacillus

Bacteroides

Fusobacterium

Prevotella

Oral

(Phylum)

Firmicutes

Proteobacteria

Bacteroidetes

Spirochaetes

Fusobacteria

Actinobacteria

Figure 2. The microbial community of feline nasal, oral and gut environments (adapted from [69], [89], [37], [114], [115], and [117])