Role of gut microbiota in dog and cat's health and diseases

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Abstract

Mammalian gastrointestinal tract is colonized by a large number of microorganisms, known as gut microbiota, that play a key role in the physiological and pathological states. In particular, the gastrointestinal tract of dogs and cats harbors a complex and highly biodiverse microbial ecosystem. Recent studies see it involved in a wide range of life processes, including energy needs, metabolism, immunological activity, and neuro-behavioral development. This review focuses on the role of the microbiota on the health of pets and will discuss changes that occur in the disease.

Keywords: Cat, Dog, Gut, Microbiota.

Introduction

Bacteria, archaea, fungi, protozoa, and viruses, which have a positive role on life processes and host health, inhabit the gastrointestinal tract (GI) of cats and dogs (Suchodolski, 2011). Different body sites present their own microbiota and gut microbiota is the largest one. It is composed by 10^{13}–10^{14} cells (more than 1,000 bacteria species) and its genome is 150 times larger than most of animals' genome (Simpson *et al.*, 2002).

Depending on pH value and residence-time, there is a different concentration of bacteria in different GI tracts (Savage, 1977). Recently, the development of new molecular technologies, such as next-generation sequencing (NGS), has allowed understanding the complexity and diversity of gut-microbial communities (Junhyung *et al.*, 2017). Molecular-phylogenetic analysis of the bacterial 16S rRNA gene has created a more detailed inventory of bacteria groups present in the bowel. Although there are differences of GI inter- and intra-species, 16S rRNA gene sequencing has shown that more than 90% of bacteria belong to two phyla: *Bacteroidetes* and *Firmicutes* (Simpson *et al.*, 2002; Ritchie *et al.*, 2008). The phylum *Firmicutes* includes *Clostridium* clusters, which include *Ruminococcus* spp., *Faecalibacterium* spp., and *Dorea* spp. These bacteria, *Bacteroidetes* and *Actinobacteria* can produce short-chain fatty acids (SCFAs), the essential energy source for colonocytes. Moreover, they help to maintain the epithelial barrier by strengthening tight junctions, to regulate intestinal motility and to stimulate the production of anti-inflammatory compounds. Because of its important functions, the gut microbiota is frequently defined as "forgotten organ" (O'Hara and Shanahan, 2006).

Many research studies on mammalians have highlighted how the microbiota is involved in many host life-processes, such as energy requirement, metabolism, immunologic activity, and neuro-behavioral development. Changes in gut-microbiome are associated with diseases, including inflammation, obesity, metabolic syndromes, and mood disorders. A balanced microbial ecosystem is crucial for host health and homeostasis (Guard *et al.*, 2017).

Role of the physiological gut microbiota

The interaction between gut microbiota, its host, and other somatic cells regulates many functions, such as digestion, host metabolism, vitamins synthesis (vit. K and complex B), biotransformation of bile acids, xenobiotics metabolism, correct maturation of gastrointestinal cells, and defense against pathogenic bacteria (Steiner and Ruaux, 2008). Therefore, the microbiota can be defined as a metabolically active "organ."

Genetic diversity of microbiota in the gut guarantees the presence of many enzymes and biochemical pathways that otherwise the host does not possess.

The bacteria metabolic activities produce energy and substrates useful both to bacteria for their proliferation and to the host. The main activity is the fermentation of alcohols and non-digestible carbohydrates (starch, inulin, cellulose, hemicellulose, pectin, and gum) (Schmitz and Suchodolski, 2016). It occurs in the colon (Bell *et al.*, 2008) and it causes the production of gasses and SCFAs, acetate, propionate, and butyrate (60:25:15), which give energy to the intestinal epithelium and other tissues (Wong *et al.*, 2006). SCFAs synthesis leads to a reduction of intestinal pH value, which is the first sensory line of defense and it supports growth, proliferation, and differentiation of epithelial intestinal cells. Commensal bacteria defend the host from pathogens, producing bacteriocins and colicins and competing for nutrients and ecological benefits (Liévin-Le Moal and Servin, 2006). Moreover, when SCFAs are absorbed through the intestine, they allow the re-absorption of Na+ or K+ ions (Fleming *et al.*, 1991).

Another important role of microbiota is the transformation both of primary bile acids (Colic acid and chenodeoxycholic acid) and secondary bile acids

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(deoxycholic and lithocholic), which are essential in the absorption of dietary fats and liposoluble vitamins in the gut (Staggers *et al.*, 1982).

Furthermore, commensal bacteria have a fundamental role on the induction, shaping, and function of the host immune system, which in turn is important in the development of the physiological gut structure and the identification of pathogens from commensal bacteria. This relationship allows the defensive responses against pathogens being effective and it regulates the pathways involved in the tolerance of innocuous antigens. The ability of microbes to set the immunological tone of tissues, both locally and systemically, requires tonic sensing of microbes and complex feedback loops between innate and adaptive components of the immune system (Suchodolski and Simpson, 2013). The intestinal mucosa is an interface between the immune system and the external environment. Results of several studies have shown that germ-free animals have a lower density of lymphoid cells in the intestinal mucosa and a lower quantity of immunoglobulins (Ig) in serum. The exposure of these animals to commensal microbes induces a rapid increase of lymphocytes and Ig in the bowel and of Ig in the serum (Capurso, 2016). Development and regulation of lymphocytes T helper type 1 and 2 are influenced by bacteria, which can suppress NF-kB through the production of butyrate and acetate and can act on the receptors linked G-protein. There is a dynamic relationship between the immune system and microbiota. Intestinal mucosa, with Toll-like receptors, induces fall signal and provokes the production and the release of inflammatory mediators (cytokines, phagocytes) (Schmitz and Suchodolski, 2016; Garraway *et al.*, 2018).

Dysbiosis of the gut microbiota in animal disease

Hippocrates used to say: "bad digestion is the root of all evil." This sentence has held up to modern scientific scrutiny.

As previously described, the microbiome participates in the vital physiologic and immunologic process, but it is unclear how it directly affects the pathogenesis of a disease state.

In healthy conditions, the crosstalk and the cross-regulation between the host and the microorganisms create a homeostatic balance of bacteria, so the gastrointestinal tract remains healthy and free from an overgrowth of potentially pathogenic bacteria. In that case, there is a state of eubiosis. When this condition of homeostasis is disrupted, dysbiosis occurs. In case of dysbiosis, there is an imbalance in bacterial composition, and bacterial metabolic activities and/or bacterial distribution inside the gut change. Three types of dysbiosis can be defined as follows:

- reduction of bacterial diversity;
- loss of beneficial bacteria;
- overgrowth of pathogens.

Each of those cases is linked to other problems (Xenoulis *et al.*, 2008). A state of dysbiosis is found in a wide range of diseases, such as inflammatory bowel disease (IBD), obesity, allergy, diabetes, and autism, but it is unclear if it is a cause or a consequence. Several studies about these diseases have indicated the presence of a microbial alteration, but no consistent pattern of microbiota changes has yet been observed. In IBD dogs, Minamoto *et al.* (2014) have highlighted an increase of *Gammaproteobacteria* and a decrease of *Erysipelotrichia*, *Clostridia*, and *Bacteroidia*, using 454-pyrosequencing on fecal samples. Suchodolski *et al.* (2012) had applied the same method on the same kind of samples and they found IBD dogs characterized by an increase in *Sutterella* and *Clostridium perfringens*, and a decrease in *Blautia*, *Ruminococcaceae*, and *Turicibacter*. Even if the result of the two studies is not the same, they both indicate that a reduction in the diversity of *Clostridium* clusters XIVa and IV in dog gut microbiota are associated with IBD. The same condition has been found in human and cats IBD. These results underline the importance of those clusters of bacteria, which also produce SCFAs, in the maintenance of gastrointestinal health (Suchodolski *et al.*, 2012; Garcia-Mazcorro and Minamoto, 2013).

Manipulation of the gastrointestinal microbiome

During a state of disease, therapies applied have the purpose to change the microbiota community. Besides the diet, probiotics, prebiotics and antibiotics administration affect, and change microbiota composition, but their efficiency is not clear. Recently, it has been developed a new method that considers restoration of the colonic microflora by introducing healthy bacterial flora. It is named fecal microbiota transplantation (FMT) (Cammarota *et al.*, 2014).

The following paragraphs are going to describe the different strategies applied and the results of the recent research studies.

Prebiotics and probiotics

Use of pre- and probiotic is broadly spread in human medicine to preserve or restore a healthy condition (Sanders *et al.*, 2018). The employment of these devices is new in veterinary medicine and pet treatment.

Prebiotics are more recent and, in accordance to their first definition given in 2015, they are "*a non-digestible compound that, through its metabolization by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host*" (Bindels *et al.*, 2015).

Nowadays, several research studies report benefits from the addition of prebiotics in pets' diet. Indeed, they beneficially modulate gut microbiota and protect the animals from enteric infections.

There are some studies about their use in the domestic canine diet. Zentek *et al.* (2003) found that in dogs, an intake of 1.5% inulin could reduce fecal pH and increase *Bifidobacteria* population. Middelbos *et al.* (2010), using

16S rRNA sequencing, have shown that dogs fed with relatively small amount of dietary fiber change structure of gut microbiota, increasing the density of *Firmicutes* and decreasing that of *Fusobacteria*. Another study underlined how a dietary supplementation of fructo-oligosaccharides (FOS) induces beneficial effects, such as the growth of *Bifidobacteria*, and it improves the digestibility of several minerals in the entire GI tract in the dog (Pinna *et al*., 2018).

Few research studies about feline nutrition and use of prebiotics have been conducted. Two studies describe that the high-protein dietary intake, typical of cat diet, mainly induces the growth of fecal *Clostridium* (Lubbs *et al*., 2009; Hooda *et al*., 2013). Barry *et al*. (2010) have found that the addition of FOS to feline diet bring to an increase of *Bifidobacteria* concentration and to a decrease of *Escherichia coli* population. The same research showed how the addition of pectin in cat nutrition raises the *C. perfringens* and lactobacilli concentration (Barry *et al*., 2010). Table 1 shows a summary of the studies on the effect of prebiotics on dog and cat.

Thousands of years ago, human beings discovered benefits deriving from fermented food (Di Gioia and Biavati, 2018). Therefore, the employment of probiotic is more ancient than that of prebiotic. They are defined as "*live microorganisms that when administered in adequate amounts confer a health benefit on the host*" (FAO/WHO, 2001). Both in human and pets, probiotics have many beneficial effects on host health, especially in the modulation of the immune system and in stress state. The use of probiotics in livestock is broadly spread, whereas in pet nutrition is still developing (Di Gioia and Biavati, 2018).

Sauter *et al*. (2005) conducted an *ex vivo* study in dogs with chronic enteropathies. This research confirmed the positive effect of a probiotic cocktail, containing three different *Lactobacillus* strains, on cytokine expression, mainly through the regulation of T-cells. Rossi *et al*. (2014) found that the administration of probiotics promotes the intensification of T-cell expression. In this study, a probiotic cocktail, similar to that of human VSL#3, was formulated for dogs with IBD. Data highlighted an increase of T-cell's target, a decrease of *Faecalibacterium* population, and recovery from the dysbiosis.

From weaning to 1 year of age, puppies' diet is often supplemented through addition of probiotics. Indeed, it has been demonstrated the presence of adjuvant effects of *Enterococcum faecium* at both intestinal mucosal level and systemic level. It is possible that these effects are relevant in the improvement of the protective immune response during the weaning period (Benyacoub *et al*., 2003).

Table 2 shows a summary of the studies on the effect of prebiotics treatment.

Antibiotics

Dogs and cats with chronic enteropathies are treated with antibiotics when diet change does not work (Makielski *et al*., 2018). The most common molecules

Table 1. Studies of prebiotic treatment in cat and dog.

Animal	Prebiotic treatment	Effect of treatment	References
Cat	Diet with the supplement of FOS and cellulose	Increase of *Bifidobacteria* and decrease of *Escherichia coli*	Barry *et al*., 2010
Cat	Diet with the supplement of FOS and pectin	Increase of lactobacilli and decrease of *Clostridium perfringens*	Barry *et al*., 2010
Cat	Diet with high intake protein	Increase fecal *Clostridium*	Lubbs *et al*., 2009
Dog	Dry diets, one supplemented with 3% chicory (1.5% inulin)	Reduction of fecal pH and increase *Bifidobacteria* population	Zentek *et al*., 2003
Dog	diet with 7.5% beet pulp (60% total dietary fiber, ~4:1 insoluble:soluble fiber)	Increase the density of *Firmicutes* and decrease that of *Fusobacteria*	Middelbos *et al*., 2010
Dog	Diet with the supplement of FOS	Growth of *Bifidobacteria*	Pinna *et al*., 2018

Table 2. Summary of the studies on the effect of probiotics treatment.

Animal	Disease	Probiotic treatment	Effect of treatment	References
Dog	Chronic enteropathies	Administration of mixture of probiotic bacteria: *Lactobacillus* spp. (two *Lactobacillus acidophilus* strains (NCC2628, NCC2766), one *Lactobacillus johnsonii* strain (NCC2767)	Beneficial effects on cytokine expression mainly through regulatory T-cells	Sauter *et al*., 2005
Dog	IBD	Administration of VSL#3	Significant decrease in clinical and histological scores and decrease in CD3+ T-cell infiltration.	Rossi *et al*., 2014

used are metronidazole and tylosin (Simpson, 2011). Metronidazole acts on bacteria and protozoa. Usually, it is used simultaneously to nutritional therapy and a diet change, so it is difficult to know its real effect (Jergens *et al*., 2003; Craven *et al*., 2004; Münster *et al*., 2006; Garcìa-Sancho *et al*., 2007).

Tylosin is employed in the treatment of tylosin-responsive chronic diarrhea, which usually affects adult dogs. Many studies underline its efficiency (Westermarck *et al*., 2005; Kilpinen *et al*., 2011) but its mechanism of action is still unknown.

Enrofloxacin is another antibiotic used in enteropathies. It is a fluoroquinolone useful in the therapy of granulomatous colitis of Boxer (Davies *et al*., 2004).

The use of antibiotics in the treatment of certain diseases has contraindications. Indeed, abundant employment of antibiotics may reduce beneficial bacteria population, facilitate the increase of potential pathogens population, and promote the phenomenon of antimicrobial resistance. Some researchers have shown that antibiotics actually modify the microbial composition decreasing bacteria diversity, so they contribute to the onset of dysbiosis (Grønvold *et al*., 2010). Over the past years, the phenomenon of antimicrobial resistance has been increasing and has assumed a great importance. The close cohabitation of human with pets can cause the transfer of antimicrobial resistance to bacteria that affect also humans (Damborg *et al*., 2008).

Fecal microbiota transplantation (*FMT*)

FMT is a medical non-pharmacological experimental treatment, which aims to restore microbial diversity. It consists in a procedure of transplant of fecal matter from a healthy donor to a patient affected by a gastrointestinal disease that does not respond to common therapies. It is made using colonoscopy, endoscopy, sigmoidoscopy, or enema (Cammarota *et al*., 2014). Fecal transplant was an ancient procedure, firstly documented in the 4th century China. However, it has gained popularity during recent years. In human medicine, this procedure is used in the therapy of *Clostridium difficile* infection, with an efficiency of 87%–90% (Bakken *et al*., 2011), and in the treatment of IBD (Kelly *et al*., 2015).

Research studies about the FMT application in veterinary medicine are few, even if they dare are promising. This treatment has been employed in dogs affected by gastrointestinal disease not respondent to common therapy, such as IBD and *Clostridium perfringes* infection. A recent case report describes the use of FMT in the treatment of one dog with refractory IBD and one cat with non-responsive chronic enteropathy. In both cases, fecal consistency is improved within 24 hours post FMT (Weese *et al*., 2001).

Even if FMT seems to be relatively sure and an efficient therapy for the treatment of dysbiosis, more clinical research studies are necessary to understand better its mechanisms and potential risks for the patients that receive these infusions. Nowadays, only limited data are available.

Conclusion

The gut microbiota is essential for the health of all mammals because it participates in the host vital physiological processes and development.

Alterations of the intestinal microbial populations are associated with a variety of gastrointestinal and systemic illnesses. Therefore, making attention to gut microbiota could be useful in the diagnosis and therapy procedures.

Future research studies should clarify the mechanisms that regulate the interactions between the microbiota and the host. More studies have to be done about the use of probiotics, prebiotics, and FMT in the restoration of a state of eubiosis. It is important to understand better the mechanisms of action and the duration of efficiency of the different treatments.

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