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Microbiota and probiotics in canine and feline welfare

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ABSTRACT

Dogs and cats have been cohabiting with us for thousands of years. They are the major human companions. Today, dogs and cats live in urban areas. Cats and most dogs are on high carbohydrate diets and face similar life-style challenges as the human beings. The health and well-being of companion animals, just as their owners, depends on the gut microbes. Providing a proper care and nutritionally balanced diet to companion animals is recognised as a part of our responsibility to maintain the health and well being of our pet. However, as microbiota differences may facilitate exposure to pathogens and harmful environmental influences, it is prudent to search for novel tools to protect dogs and cats and at the same time the human owners from pathogens. Specific probiotic strains and/or their defined combinations may be useful in the canine and feline nutrition, therapy, and care. Probiotic supplementations have been successful in the prevention and treatment of acute gastroenteritis, treatment of IBD, and prevention of allergy in companion animals. New challenges for probiotic applications include maintenance of obesity and overweight, urogenital tract infections, *Helicobacter* gastritis and parasitic infections. The probiotics of human origin appear to be among the new promising tools for the maintenance of pets' health. However, the host-derived microorganisms might be the most appropriate probiotic source. Therefore, more controlled trials are needed to characterise new and safe probiotic preparations with an impact on general health and well being as well as health maintenance in dogs and cats.

1. Introduction

Pet population is increasing especially in westernised countries, and dogs and cats are the major human companions. Both have been domesticated and are co-existing with humans for thousands of years. Most of pet owners consider the animals to be family members, and other consider their pet as companions [1]. Domestic dogs and cats still live in conjuction with humans benefiting from each other. Mutual interest has evolved into companion animals being a stable part of human life and therefore, the health and well being of pets have increasingly raised interest during last decades.

Dogs and cats are carnivores with a history of high protein diets [2,3]. Today, cats and most dogs are on high carbohydrate diets living in urban areas and thus face similar life-style challenges as the human beings. The health and well-being of companion animals, just as their owners, depends on the gut microbes. Microbiota composition and activity have been associated with several diseases in both the animals and their owners [4–6]. This interrelationship would benefit from more exact knowledge on microbiota in pets and information on how the microbiota affects the health of the pets in the long-term.

A major part of the animal health derives from the intestine. The 'healthy gut' is known to be crucial for the physiology and well being of the host. The gastrointestinal tract (GIT) harbours a complex microbial community. This microbial ecosystem acts in several ways, affecting both absorption and metabolism of nutrients, trophic and protective functions of the host. Any disturbances within the gut microbiota may lead to the development of a multitude of diseases and disorders e.g. diarrhoea, allergies, obesity, and stress symptoms [5].

Knowledge on the canine and feline intestinal microbiota is still expanding. Most studies on microbial community in the dog and cat GIT implemented in traditional microbiological techniques, however several recent reports characterised microbiota using novel molecular methods such as qPCR, FISH or 16S rRNA sequencing [7,8]. To balance the disturbed microbiota and to

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combat infections, different therapeutic agents have been proposed, among them probiotics. However, published papers on probiotic applications in companion animals are greatly limited compared to data regarding humans. The aim of this review is to describe the current knowledge on the gut microbial community and advances in probiotic therapy in dogs and cats.

2. Canine and feline microbiota

All animals harbour a vast and complex community of microorganisms. Dogs and cats have high numbers of microorganisms in the GIT which in fact outnumber those living in human gut. However, both dogs and cats have distinct bacterial species that differ between each other and also vary in different dog and cat species, various gut niches and geographical areas. Microbial diversity and concentration increase along the length of the gastrointestinal tract. The predominant bacterial phyla in the colon and faeces of both dogs and cats are represented by *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Fusobacteria* as well as *Eubacterium* in cats. The microbial differences between dogs and cats are demonstrated in the microbial groups and species levels. Molecular fingerprinting has also revealed that every individual pet has a unique and stable microbial ecosystem [9].

Molecular analysis using 16S rRNA sequencing revealed that *Firmicutes*, *Fusobacteria*, *Bacteroidetes*, and *Proteobacteria* commonly inhabit the canine GIT [7,10]. Clostridia predominate in the duodenum and jejunum and are highly abundant in the ileum and colon [11], while *Fusobacteria* and *Bacteroides* bacteria are the most abundant in the ileum and colon [7,11]. Fungi, such as *Ascomycota*, *Basidiomycota*, *Glomeromycota*, and *Zygomycota* have been identified as well [10].

Lactobacilli inhabit commonly all parts of the dog intestine [11], ranging from 10^4 to 10^8 CFU/ml and among them *Lactobacillus acidophilus* is dominant [12]. *Lactobacillus fermentum*, *Lactobacillus rhamnosus*, and *Lactobacillus salivarius* are reported being a part of health canine intestine [13]. Other canine lactobacilli are represented by *Lactobacillus murinus* and *Lactobacillus reuteri* [12], *Lactobacillus animalis*, *Lactobacillus sanfranciscensis* and *Lactobacillus paraplantarum* [14].

Lactobacilli found in cats are typical intestinal lactobacilli, e.g. *L. acidophilus*, *L. salivarius*, *Lactobacillus johnsonii*, *L. reuteri* and *Lactobacillus sakei*, which can be seen in other animals, including human [15]. However, *Lactobacillus* distribution, similar to bifidobacteria, varies between individuals.

Bifidobacteria have been difficult to characterise using pyrosequencing due to methodological difficulties, although the microorganisms are well known to be beneficial in several animals and especially in human infants. Jia and collaborators reported that dogs contain over 10^8 cells/g of bifidobacteria in their faeces, as determined by fluorescence *in situ* hybridisation (FISH) [8]. This is consistent with the reports studied by culturing [16,17]. Both animal type (e.g. *Bifidobacterium pseudolongum* and *Bifidobacterium animalis*) and human type (e.g. *Bifidobacterium catenulatum* and *Bifidobacterium bifidum*) of bifidobacteria have been found in dog faeces [18–20]. Bifidobacteria were major proportions in healthy cats with cell number of 9.34 (log10 cells/g faeces), as determined by FISH. Significantly lower numbers of bifidobacteria have been found in cats with IBD [21]. Ritchie and co-workers [15] found several human-type and animal-type bifidobacteria in cats using clone library combined with *Bifidobacterium* group-specific primer set. They have also found that individual cats contained several unique *Bifidobacterium* species.

A study on pet cat oral microbiota indicated *Proteobacteria* being dominating (75.2%) leaving amounts of *Bacteroides* (9.3%), *Firmicutes* (6.7%), unculturable (2.7%), *Spirochaetes* (1.8%), *Fusobacteria* (1.3%), and *Actinobacteria* (0.6%) low [22].

Feline faeces have been found to contain high numbers of obligate anaerobes, and such quantities are considered abnormal in dogs and humans [23]. Based on the analysis of 16S rRNA, it was reported that the feline GIT is often represented by five phyla where *Firmicutes* dominate (68%), followed by *Proteobacteria* (14%), *Bacteroidetes* (10%), *Fusobacteria* (5%), and *Actinobacteria* (4%), [15]. However, a more recent study using the metagenomic approach revealed that the feline gut microbiota is predominated by *Bacteroidetes*/*Chlorobi* group bacterial phylum comprising around 68% of total classified diversity and followed by *Firmicutes* (~13%) and *Proteobacteria* (~6%) respectively, while known *Archaea*, fungi (*Ascomycota*), and viruses represent minor communities in the overall microbial diversity [10,24]. Minamoto and collaborators [25] summarised the studies on feline small and large intestine microbiota showing in detail microbiota to genus level. Most studies have been conducted in shorthair cats [8] which leaves much of the microbiota of other pet cats in different breeds to be studied more in detail. Thus, the composition appears to be distinctly different from both canine and human studies. Since different techniques may create bias from one report to another and the result, more studies are needed to uncover the details of variation within the canine and feline community and also between individual animals.

An increasing numbers of reports have changed the old dogma suggesting initiation of microbiota development at birth. These reports clearly demonstrate that humans and mice are colonised by microbiota already in utero and prior to birth [26,27]. There are no studies on canine and feline microbial colonisation prior to delivery, however similar possibilities cannot be excluded since both are mammals and share similar physiological and some anatomical patterns with humans.

The skin microbiota plays an important role in skin function, potentially enhancing the skin barrier and reducing the colonisation by potentially pathogenic microorganisms. One recent study using pyrosequencing demonstrated that canine skin is inhabited by rich and diverse microbial communities. Healthy dogs have high individual microbial variation between samples collected from different skin sites. Also higher species richness and microbial diversity are observed in the samples from haired skin when compared to mucosal surfaces or muco-cutaneous junctions. The most abundant phyla and families identified in the different regions of skin and mucosal surfaces are members of *Proteobacteria* and *Oxalobacteriaceae*. On the other hand, the skin of allergic dogs is characterised by lower species richness when compared to the healthy dogs, with lower proportions of the β-Proteobacteria *Ralstonia* [28].

The composition of canine and feline microbiota is shown in Fig. 1a, b.

3. Microbial interaction between pets and owners – is there a connection?

Gastrointestinal disorders are one of the main reasons owners bring their pets to the veterinarian for healthcare. In addition, specific bacterial canine enteropathogens such as *Salmonella* and *Campylobacter* are well-documented zoonoses [4,29] being a challenge for pet owners, veterinary and medical care. Dogs may harbour and shed cysts of protozoan parasite *Giardia lamblia* without showing clinical signs [30] and ascariasis. Both are common potentially zoonotic diseases leading to human infections [31]. The enteric zoonotic agents in domestic cats are also important. A study by Tun and collaborators [24] identified a range of potential enteric zoonotic pathogens (0.02–1.25%) and genes involved in antimicrobial resistance (0.02–0.7%) in feline excrements which

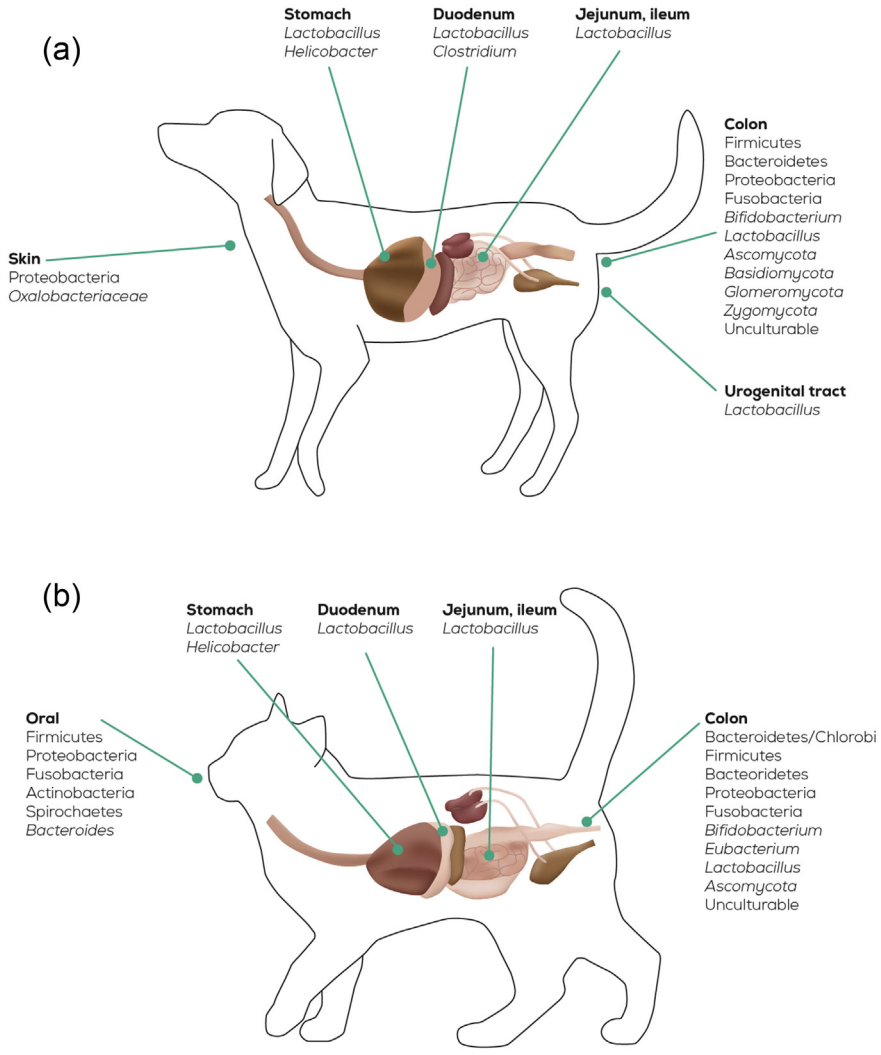

Fig. 1. The canine (a) and feline (b) gastrointestinal tract and its dominant microorganisms. Skin microbiota included.

highlight the potential for transmission of opportunistic infections to human.

Recent studies on pet and human microbial interaction demonstrate that having a dog at home increases the shared skin microbiota between cohabiting adults. In addition, dog-owners adults share more 'skin' microbiota with their own dogs than with other dogs [32]. Studies suggest that exposure of infants to farm animals is associated with a decreased risk of development of asthma later in life [33]. Finally, it has been demonstrated that perinatal pet exposure affects the compositional differences in the gut microbiota and protects children from early wheezing [34]. Reverse zoonotic occurrence has been acknowleged lately and could be interpered throughout microbiotal exchange in human–animal relationship [35].

4. Effect of diet on microbiota

The nutritional composition and contents of feed is known to significantly influence gastrointestinal function, microbiota composition and their metabolic products in the animal gut. Companion animal diets have been evolving from hunted food and table scraps to scientifically and nutritionally balanced target formulated foods. Table scraps do not fulfil the basic metabolic needs of companion animals. Well made feeds offer a good source of all necessary nutrients, vitamins, minerals, and other components when feed quality is adequate. Often, canine and feline feed preparations are enriched with probiotics offering a microbiota modulating and well-being targeted functional feeds.

A pilot study involving five beagle dogs fed different diets revealed that high-carbohydrate starch based feed leads to decrease in faecal ammonia. On the other hand, high-protein greaves-meals (a by-product which is produced when melting raw fat from e.g. pigs), commonly thought as low-grade meat meal, lead to higher pH, decrease in propionic and acetic acids and increase in branched-chain fatty acids and valeric acid and changes in faecal total volatile fatty acids, higher faecal canine calprotectin concentration which was associated with increased valeric acid concentration. In addition, all studied dogs fed high-protein greaves-meals developed diarrhoea [36].

4.1. Prebiotics in canine and feline nutrition

Only a few studies have been reported on prebiotics and canine feeds suggesting that dogs may benefit from the addition of prebiotics to their diet. Healthy dogs fed a diet supplemented with chicory, a fermentable oligosaccharide, demonstrate firmer faecal

consistency, a lower faecal pH, and increased levels of bifidobacteria and decreased *Clostridium perfringens* counts in their stools as compared to animals on a protein-rich diet [37]. On the contrary, feeding dogs with low level of dietary fibre (beet pulp), 7.5 g/day for 2 weeks leads to decrease in *Fusobacteria* and increase in *Firmicutes* [38].

New types of prebiotics are being constantly developed and they seem to be a promising additive in animal feed. The consumption of new fermentable prebiotic polydextrose by dogs was reported to increase faecal acetate, propionate and total SCFA concentrations, while it decreased faecal pH and indole. In addition, faecal *C. perfringens* decreased but *Escherichia coli*, lactobacilli and bifidobacteria remained unaffected in animals [39]. Clearly, more extensive research should be conducted to provide detailed coverage of the canine microbiome, to assess the effects of age, genetic background or food environment on its composition and activity. Such studies would provide a better understanding on the associations between intestinal microbiota and disease and provide new possibilities of modulating the health of dogs through diet.

Feline diet which is characterised by high-animal-protein supplemented by low-carbohydrate plant-based additives has been found to favour the growth of faecal *Clostridium*, *Faecalibacterium*, *Ruminococcus*, *Blautia*, and *Eubacterium* in kittens, and affect circulating hormones and metabolites that may be important in terms of satiety and host metabolism. On the other hand, kittens fed with moderate-protein, moderate-carbohydrate diets, contain greater amounts of faecal *Actinobacteria*, *Dialister*, *Acidaminococcus*, *Bifidobacterium*, *Megasphaera*, and *Mitsuokella*, as well as lower amounts of *Fusobacteria* and such changes may have a beneficial effect on gut health [40]. Similarly, earlier study found that cats fed diet containing high protein concentration show lower bifidobacterial and higher *C. perfringens* counts as compared to animals fed moderate-protein diet [41].

Addition of prebiotics to feline diet may positively affect gut microbial populations. Cats fed diet supplemented with FOS (4% of diet) had increased bifidobacterial concentrations while counts of *E. coli* decreased. Presence of pectins (4% of diet) in feline diet increased *C. perfringens* and lactobacilli concentrations. These studies also suggest that while the cat is a carnivore, its gut microbiota is similar to omnivores in microbial phylogeny [42–44].

Thus, incorporating prebiotics in pets diet may beneficially modulate gut microbiota and intestinal health and possibly protect the animals from enteric infections.

4.2. Obesity and overweight in companion animals – a global issue

Obesity and overweight are major health problems in humans as well as in pets. Inappropriate and high-caloric diet, sedentary lifestyle and limited physical activity in urbanised pets are only few factors that lead to overweight in companion animals.

Recent studies demonstrate that around 38% of dogs are overweight [45]. The prevalence of canine obesity is estimated to be 39% in France [46] and 50% in the UK [47]. A recent study on the microbiota in obese dogs revealed that the phylum *Actinobacteria* and the genus *Roseburia* were significantly more abundant in obese dogs compared to lean animals [48]. Clearly more studies are needed to reveal the microbial composition and activity associated with overweight and the shifts in microbial community structure in canine obesity.

The problem of obesity also affects cats. However, to our knowledge, no studies on microbiota in obese cats have been conducted.

5. Probiotics for dogs and cats

Antibiotics used commonly to treat certain diseases can reduce the population of beneficial bacteria and increase the levels of potentially harmful microorganisms and also lead to antimicrobial resistance [49,50]. In addition, antibiotic therapy has been shown to have a long-term effect on intestinal microbiota [51,52]. Microbes with antimicrobial multiresistancy, such as ampicillin- and tetracycline-resistant enterococci, may transfer from pets to pet owners occuring even in hospital infections [49,53,54]. An experimental study evaluating the effect of macrolide antibiotic tylosin on microbial diversity in the canine small intestine using 16S rRNA gene sequencing revealed decrease of *Fusobacteria*, *Bacteroidales* and *Moraxella*, while the proportions of *Enterococcus*-like bacteria, *Pasteurella*, *Dietzia*, and *Escherichia* increased. Microorganisms belonging to *Spirochaetes*, *Streptomycetaceae*, and *Prevotellaceae* were undetectable at the end of antibiotic challenge [55].

The origin of probiotics forms the basis to prevent and treat various disorders and diseases in farm animals. Recently, probiotic products aimed for pets, especially dogs and cats, have also gained popularity among their owners as science provides the first products with proven efficacy. Therefore, supplementing animal diet with defined and undefined probiotics [56] seems to be an essential way to help maintain and promote the optimal GIT health and well-being of pets as companion animals.

Probiotic is defined as 'live microorganism which when administered in adequate amounts confers a health benefit on the host' [57]. The definition has been recently reformulated by an international consensus panel and also different product classes have been included [56]. The majority of probiotic strains for humans and animals belong to lactic acid bacteria (LAB) and for humans bifidobacteria [58]. There are several possible modes of action of probiotics on the host and they include: production of antibacterial compounds, competition for nutrients and adhesion sites with potential pathogens, alteration of microbial metabolism, stimulation of immunity among the others [59,60]. Possible benefits of the probiotic use in pets include: modulation of the immune system, help in stress maintenance, protection from infections caused by enteropathogens, increased growth and development, control of allergic disorders and recently also obesity (Fig. 2).

6. Origin of probiotics, survival in GIT and effect on microbiota

The probiotic characteristics of microorganisms are linked to host specificity [61]. Thus, for successful use as a probiotic, the bacterial species should be of host intestinal origin. Unfortunately, most of the probiotics for companion animals are not originally derived from the canine or feline GIT microbiota. The canine and feline intestine is, however, rich in microorganisms with probiotic potential [14]. In addition, few studies have been published on the quality of probiotic products for pets and probiotic survival through the GIT.

Even though lactobacilli form a small part of the canine GIT microbiome, they are wide spread, and several isolated LAB strains including *Lactobacillus* species demonstrate *in vitro* antimicrobial activity [13,14,62,63] and survive in and dominate the small-intestinal LAB microbiota during feeding and have the ability to modify the intestinal microecosystem [64]. Further, three canine lactobacilli, *L. fermentum* VET9A, *Lactobacillus plantarum* VET14A, *L. rhamnosus* VET16A and their mixture demonstrated good adhesion ability to canine intestinal mucus and were able to exclude the adhesion of common enteropathogens (*Enterococcus canis*, *C. perfringens*, *Salmonella enterica* ser. Typhimurium) from the canine intestinal mucus *in vitro* [65,66]. Thus, specific LAB including

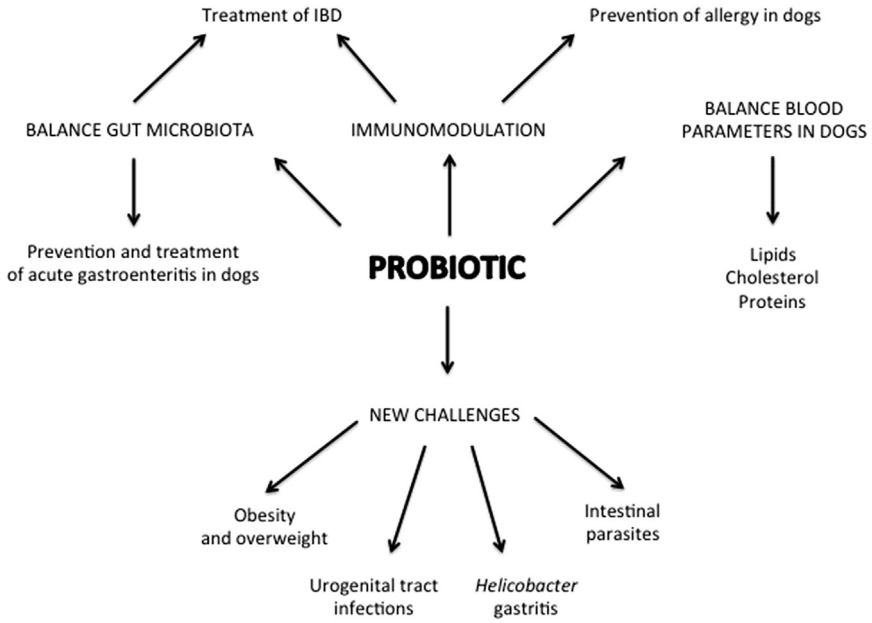

Fig. 2. Documented health benefits and new challenges of probiotics in companion animal health.

lactobacilli may serve as potential probiotics for canine use.

A new potential probiotic *L. fermentum* AD1 strain isolated from canine faeces expressed *in vitro* survival by pH 3.0 after 3 h (86.8% survival) and in the presence of 1% bile (75.4% survival). The strain adhered to the canine intestinal mucus. Given to 15 healthy animals for 7 days at a dose of 10^9 CFU/mL, it increased faecal lactobacilli and enterococci levels in the canine faeces [67]. Also a canine-originated probiotic strain *Enterococcus faecium* EE3 administered for 1 week to 11 healthy dogs at a dose of 10^9 CFU/mL was able to survive the GIT passage and persisted in faeces for 3 months after cessation of its administration at a level of 6.83 ± 0.95 log CFU/g. This strain was able to decrease staphylococci and *Pseudomonas*-like bacteria and increase concentration of other LAB while *E. coli* levels remained unaffected [68].

In a study evaluating the quality of *L. acidophilus* DSM13241 as a feed additive (10^9 CFU/mL for 4 weeks) to healthy adult dogs, the probiotic was found to be stable in dry feed. It also survived transit through the canine GIT, increased numbers of lactobacilli and decreased clostridia in faeces, and resulted in improved blood and immune parameters [69]. In addition, a canine originated *L. animalis* LA4 (10 day study with a daily dose of 0.5×10^9 CFU) as well as *B. animalis* AHC7 (a six-week trial with a daily dose of 1.5×10^9 CFU) have been reported to reduce *Clostridium difficile* count in dogs [70,71].

A study evaluating the ability of *L. rhamnosus* GG (LGG) to survive gastrointestinal transit in dogs demonstrated that the LGG recovery after discontinuation of a probiotic administration was dose dependent. LGG given at the dose of 5×10^{11} CFU per day was still recovered 4 days after cessation of administration. Also no side effects of the LGG administration were found [72].

In a study conducted on healthy dogs, the 18-day application of the probiotic *E. faecium* NCIB 10415 at a dose of 9.2×10^9 CFU increased counts of *Salmonella* and *Campylobacter* while clostridial levels were significantly reduced [73]. Thus, the effect of novel probiotics should be tested with caution.

Canine GIT microbiota may also be a source of bifidobacteria with a probiotic potential. New canine (from 14 healthy German Shepherd Dogs) isolates of *B. animalis* ssp. *lactis* survived in the simulated *in vitro* digestion assay, were resistant to low pH and bile salts, and demonstrated strong auto-aggregation activity [18]. Thus, these could be further tested as a potential novel probiotics for dogs.

Similar studies were performed in 15 healthy adult cats. The probiotic strain *L. acidophilus* DSM13241 given at a dose of 2×10^8 CFU/d for 4.5 weeks was able to alter the balance of gastrointestinal microbiota by increasing numbers of lactobacilli and decreasing clostridia and *Enterococcus faecalis* microbiota. In addition, the probiotic administration decreased faecal pH, plasma endotoxin concentrations and resulted in systemic and immunomodulatory changes in treated cats [74]. Thus, *L. acidophilus* DSM13241 probiotic may have the potential to improve intestinal health in cats.

The present results call for further studies on the probiotic potential of canine and feline microbiota including some human-originated strains.

6.1. Probiotic effect on the immune system

The effect of a probiotic administration on the canine immune system has little been studied. Several reports show that a dietary probiotics enhance specific immune functions in young dogs.

The administration of *E. faecium* SF68 (5×10^8 CFU/d) to dry dog feed in 14 growing puppies (from weaning to one year of age) demonstrated adjuvant effect at both mucosal and systemic levels, which could be relevant for improving protective immune responses against various infections during the critical weaning period as well as at later stages in life [75].

A symbiotic containing 5×10^9 CFU of a mixture of seven probiotic strains (*E. faecium* NCIMB 30183, *Streptococcus salivarus* ssp. *thermophilus* NCIMB 30189, *Bifidobacterium longum* NCIMB 30179, *L. acidophilus* NCIMB 30184, *Lactobacillus casei* ssp. *rhamnosus* NCIMB 30188, *L. plantarum* NCIMB 30187, *Lactobacillus delbrueckii* ssp. *bulgaricus* NCIMB 30186) and a blend of fructooligosaccharides and arabinogalactans, administered daily for three weeks in healthy dogs and cats, resulted in increased abundance of *Enterococcus* and *Streptococcus* spp. during administration. There were no changes in the major bacterial phyla as identified by 454-pyrosequencing. In addition, no adverse gastrointestinal effects

were recorded and no significant changes in gastrointestinal function or immune markers were observed during the study period [76]. The study suggested that specific synbiotic administration is safe for dogs and cats. The lack of potential effect on gut function and immune markers may have resulted from too short, only 3-week period of probiotic trial.

Just as humans, dogs may suffer from a variety of allergic diseases. Although specific probiotics appear useful in prevention and treatment of allergic diseases in humans, the studies on pets are limited. The *L. rhamnosus* strain GG (LGG) was used for the alleviation or prevention of clinical signs of atopic dermatitis in genetically predisposed dogs. The study found that early exposure of puppies to LGG appeared to reduce immunologic indicators of atopic dermatitis, significantly decreasing allergen-specific IgE in the first 6 months of life [77], and the positive effect was seen 3 years after discontinuation of probiotic administration [78].

6.2. Probiotics and clinical chemistry parameters

Although this is not an issue in canine and feline health, probiotics have a potential in controlling the lipid and protein level in companion animals. A canine strain *E. faecium* EE3 administered (10^9 CFU/mL) daily (2–3 mL) to 11 healthy dogs for a period of one week was able to reduce total lipids and proteins and the cholesterol was brought to the physiological level, i.e. in blood samples with low cholesterol values it increased to the physiological level and in those with high levels it decreased [68]. On the other hand, another canine-originated potential probiotic strain *L. fermentum* AD1 (10^9 CFU/mL) given daily (3 mL) to 15 healthy dogs for one week increased the total protein and total lipid and reduced glucose in serum of studied dogs [67]. Strompfová et al. [79] demonstrated that canine-derived strain *B. animalis* B/12 (10^9 CFU) increased organic acid levels in faeces and reduced triglyceride and albumin concentration in blood serum. Phagocytic activity of leucocytes increased as well. A greater effort is needed to uncover any physiological effects of the biochemical profiles observed and their impact on dogs health.

6.3. Probiotic effect on gut health

Acute gastroenteritis is a common disorder in small animal practice and a disturbance faced often by dog and cat owners. It may result from inadequate feeding practices, feed-borne pathogens or specific nutrient sensitivity among other non-nutrient and sometimes also unknown factors. Probiotics seem to be promising tool in alleviating gastrointestinal illnesses in pets.

The probiotic cocktail consisting of thermo-stabilised 2.85 billions live *Lactobacillus farciminis* (porcine origin), *Pediococcus acidilactici* (unknown origin), *Bacillus subtilis* (soil origin) and *Bacillus licheniformis* (soil origin) and 1.35 billions *L. acidophilus* MA 64/4E (human origin), significantly reduced the convalescence time (1.3 days in probiotic group vs. 2.2 days in placebo group) in acute self-limiting gastroenteritis in 36 dogs [80].

Discussion could be raised on the safety of *Bacillus* sp. as feed additives to cats and dogs since both pets are human companions and the bacteria could easily be transferred from animal to human during the feeding, for instance. Particularly, *B. licheniformis* has been reported to be fatal to newborn infants [81] and *B. subtilis* an opportunistic pathogen [82]. Further studies are needed on the safety concern of *Bacillus* probiotics for pets use.

Diarrhoeal diseases among dogs and cats are common in animal shelters, due to animal exposition on stress, diet change, and spread of bacterial, viral, and parasitic agents. A study evaluating the effect of *E. faecium* SF68 (2.1×10^9 CFU/g), on the incidence of diarrhoea in sheltered 182 dogs and 217 cats demonstrated that cats fed the probiotic had decreased episodes of diarrhoea as compared to placebo group. However, the probiotic had no effect on the incidence of diarrhoea in dogs [83]. The probiotic action could thus be related to specific animal target species.

Probiotic supplementation including two lyophilised *L. acidophilus* (NCC2628 and NCC2766) and one lyophilised *L. johnsonii* (NCC2667) (10^{10} CFU/g of each strain) one gram a day following specific diet for four weeks in 21 dogs with food responsive diarrhoea resulted in beneficial effects on intestinal cytokine patterns and on microbiota, diminishing numbers of enterobacteria and increasing lactobacilli in faeces. The probiotic treatment resulted in clinical improvement in all dogs [84].

Other study reported that German Shorthair Pointers dogs with non-specific dietary sensitivity (NSS) receiving probiotic *L. acidophilus* strain DSM13241, showed improved faecal consistency, faecal dry matter and defecation frequency as compared to a control group. In addition, faecal concentrations of culturable lactobacilli and bifidobacteria had a tendency to increase while the numbers of *C. perfringens* and *Escherichia* spp. had a tendency to decrease in a group receiving the probiotic [85].

Dogs and cats are known to be a reservoir of *Helicobacter*-like bacteria. These microorganisms reside in the stomach and occasionaly in the mouth of the pets [86–88] form where they could easily be transferred to humans through sniffing and licking behaviour of the animals. Suchodolski et al. [9] reported mucosa-adherent *Helicobacter* sp. being the main founding in the canine stomach. The presence of *Helicobacter* spp. has been associated with gastritis in dogs and cats [89]. The only treatment is a 'triple therapy' composed of two antibiotics and a proton-pump inhibitor [90] but at least in humans this therapy is not always successful and new alternatives are being developed [91]. Some probiotic strains, such as *L. casei* DN-114 001, showed a high success rate of eradication of *Helicobacter* in children(along with a standard treatment) at a high daily dosage of 1×10^{10} CFU [92]. Thus, probiotics could be a promising tool to combat *Helicobacter* infections in dogs and cats.

6.4. Probiotic effect on inflammatory bowel diseases

Dogs and cats may be challenged with inflammatory bowel diseases (IBD). The clinical symptoms of IBD include vomiting, diarrhoea, weight loss, and histopathologic lesions of inflammation that involves stomach, small intestine, or colon [93]. Dogs with IBD present dysbiosis of gut microbiota. Specific bacterial genera within *Proteobacteria*, including *Diaphorobacter* and *Acinetobacter* are more abundant and more frequently found in the colon of IBD dogs, while proportions of *Fusobacteria*, *Bacteroidaceae*, *Prevotellaceae*, and *Clostridiales* are found more abundant in healthy dogs [94]. Also the small intestinal microbial communities of IBD dogs differ from that of the healthy animals. The inflamed duodenum in IBD dogs is enriched with enterobacteria and clostridia but depleted in *Bacteroides* and lacks *Spirochaetes* [95].

Animals showing severe disease symptoms are usually treated with both dietary and pharmacologic interventions such as immunosuppressive drugs, and elimination diet, as well as therapeutic manipulation of the enteric microbiota through the use of antibiotics and or prebiotic supplements [93,96]. Since probiotics are capable of altering the microbiota and immune responses in the gut, they seem to be promising future tools for IBD risk reduction and nutritional therapy.

Only one study evaluated the probiotic effect in IBD treatment in dogs so far. Twenty dogs with IBD were treated with either a combination therapy (prednisone and metronidazole) or probiotic VSL#3 (a mixture of strains belonging to species *L. plantarum*, *L. delbrueckii* subsp. *bulgaricus*, *L. casei*, *L. acidophilus*, *Bifidobacterium breve*, *B. longum*, *Bifidobacterium infantis*, and *Streptococcus*

salivarius subsp. *thermophilus*) (112–225 × 10^9 CFU/10 kg) for 60 days. A protective effect of probiotic VSL#3 significantly decreased clinical and histological scores and decreased CD3+ T-cell infiltration in dogs with IBD. Probiotic-treated group demonstrated significant enhancement of regulatory T-cell markers (FoxP3+ and TGF-β+) and normalisation of gut dysbiosis [97]. The results of this study show that VSL#3 may be successful in the IBD therapy in dogs and call for further research in the area of probiotics and IBD.

IBD may affect also cats. Collecting duodenal biopsies from IBD cat patients revealed mucosa-associated *Enterobacteriaceae* being higher in cats with signs of gastrointestinal disease [98]. Inness et al. [21] reported total bacteria, *Bifidobacterium* spp., and *Bacteroides* spp. being significantly higher in healthy cats than in cats suffering from IBD where *Desulfovibrio* spp. producing toxic sulphides emerged higher in colitic cats. Bacterial count was measured by *in situ* hybridisation FISH. Dietary intervention may be an important aspect of their treatment and microbiota modulation could be a key to new dietary treatment regimens. However, studies on probiotic applications for IBD in cats are lacking.

6.5. Probiotic effect on intestinal parasites and viruses

To our knowledge, there are only few studies assessing the interactions of specific probiotics on parasites and viruses in companion animals. In a double-blinded placebo controlled study a probiotic mix of *L. fermentum* VET9A, *L. plantarum* VET14A, and *L. rhamnosus* VET16A at a total concentration of 2 × 10^9 CFU/ml was administered 100 mL twice a day for seven days to 24 dogs suffering from acute and intermitted diarrhoea. Faecal parasites (*Toxocara canis*, *Uncinaria stenocephala*, *Diphyllobothrium latum*, *Giardia* sp.) and viruses (canine parvovirus CPV, canine rotavirus CRV, canine calicivirus CaCV, canine coronavirus CCV) were monitored prior to administrating the study product. Virus account was detected also post administration at day 8 with no relevant findings (Beasley, 2014, unpublished).

A study evaluating 6-week probiotic treatment with *E. faecium* SF68 (5 × 10^8 CFU/d) to dogs with chronic naturally acquired subclinical giardiasis did not affect giardial cyst shedding or antigen content and did not alter innate or adaptive immune responses [99]. Administrating a genetically modified *L. acidophilus* for three days to cats with feline immunodeficiency virus (FIV) suggested the probiotic having an upregulating role in transcellular transport across the gastointestinal ephitelial barrier [100]. There is a recent report in human infants suggesting that specific probiotics or prebiotics may have an impact on human respiratory tract infections caused by rhinoviruses [101]. The interaction has been suggested to be modulated by binding viruses [102].

6.6. Probiotics and urogenital tract infections

Just as in women, the vaginal microbiota in healthy bitches is predominantly colonised by LAB, which may reach up to 10^8 CFU. Canine vaginal LAB have been found to exert antipathogenic activities thus have a probiotic potential [62] and could be used as strategies to combat urinary tract infections in dogs. One study evaluated oral administration of a commercial probiotic mixture containing lactobacilli (>64 bil CFU/g), bifidobacteria (30 bil CFU/g), and bacilli species (24 bil CFU/g), (strains not specified) along with a combination of yeast, enzymes, vitamins, and prebiotics, once daily for either 2 weeks or 4 weeks to 35 healthy bitches and found that probiotic mixture supplementation did not have an effect on the prevalence of vaginal LAB in studied animals [103].

Pyometra, a uterine bacterial infection can be a life threatening for dogs and cats. The treatment includes antibiotics, hormonal therapy and also surgery [104,105]. Further studies are warranted to assess e.g. whether the probiotic supplement could have a protective role against pyometra and other vaginal infections in dogs.

7. Safety issues with canine and feline probiotics

7.1. Viable probiotics versus non-viable microorganisms

Numerous commercially available probiotic products for companion animal consumption contain enterococci, mainly *E. faecium* or *E. faecalis*, which are natural habitants of the canine and feline GIT and specific strains may exert numerous benefits on the host as probiotics. On the other hand, enterococci can be pathogenic and have a notorious ability to rapidly develop, spread antibiotic resistance and favour the growth of potentially harmful microbes in animals and humans [49,106,107]. Therefore, even though no probiotic enterococcal infections have been reported in animals, theoretically they could possess certain safety risk. New research on the identification of novel strains and the assessment of functional properties are being developed [14,80,108].

There are more studies on the use of viable than non-viable forms of probiotics [78,109,110], although viability is essential for probiotics as described above. Non-viable preparations may have global economic advantages in terms of extended shelf life in non-refrigerated conditions and for storage in extreme temperature or humidity. Their use could also reduce the risks of potential microbial translocation and infection.

Recent findings demonstrate positive *in vitro* effect of three probiotic canine-originated lactobacilli (*L. fermentum* VET9A, *L. plantarum* VET14A, and *L. rhamnosus* VET16A) against canine model enteropathogens when probiotics were used in viable and non-viable forms. In addition the inactivated forms of probiotic strains were also able to adhere to dog mucus *in vitro*. Thus, non-viable probiotics have a potential as a successful probiotic feed additives in canine diet [65,66]. The *in vivo* study using non-viable forms of probiotics are also promising, although preparation method of non-viable form might have an impact on probiotic potential. Kanasugi and colleagues [111] demonstrated that oral administration of a heat-killed *E. faecalis* (FK-23) stimulated non-specific immune responses in healthy dogs. A bacterial enterovaccine Colifagina which contains deactivated whole bacteria and lysates of *E. coli* 01, 02, 055, 0111, *Bacillus pumilus*, *Morganella morgani*, *Alcaligenes faecalis*, *Shigella flexneri*, *B. subtilis*, *E. faecalis*, and *Proteus vulgaris* and is used in human medicine to treat intestinal symptoms, has been shown to improve symptoms and faecal consistency in five dogs with recurrent episodes of diarrhoea. Authors reported that such improvement lasted as long as the dogs were monitored for diarrhoea, i.e. up to one month after discontinuation of the probiotic treatment [112]. Thus, non-viable forms of probiotics could be a good alternative to live probiotic cells, but the current definition identifies only viable microorganisms as probiotics [56,57]. Nevertheless, more research on non-viable forms of probiotic bacteria could be useful to assess their antipathogenic and physiological effects on canine and feline health.

7.2. Quality and stability of probiotic products

It has been demonstrated that the production and manufacturing methods and the food carrier may influence the original properties of probiotics [113]. Since different sources of the same probiotic may significantly alter strain properties, the outcome of intervention studies may be questioned. Recently, we have shown that growth media may significantly affect the adhesive ability of canine probiotics lactobacilli to dog mucus [66]. Their impact on enteropathogen adhesion to canine mucus was also altered depending on the growth media used to cultivate probiotics

[65]. Thus, the growth conditions and media should be carefully considered for quality control of existing probiotics and for identification of new probiotics for companion animals, since even small changes in properties may significantly influence the outcome with different impact on host health. In addition, the storage stability of non-viable forms of microorganisms may provide new possibilities of developing nutritional supplements for companion animals.

8. Conclusions

Providing a proper care and nutritionally balanced diet to companion animals is recognised as a part of the duty animals' or pet owners' responsibility to maintain the health and well being of the animal. However, as microbiota differences may facilitate exposure to pathogens and harmful environmental influences, it is of importance to search for novel tools to protect dogs and cats and at the same time the human owners from pathogens. There is therefore a need for development of products aimed at improving the well-being of companion animals and probiotics are excellent candidates. The current evidence suggests that specific probiotic strains and/or their defined combinations may be useful in the canine and feline nutrition, therapy and care. The probiotics of human origin appear to be among the new promising tools for the maintenance of pets' health. However, the host-derived microorganisms might be the most appropriate probiotic source. More controlled trials are needed to both identify and characterise new specific probiotic preparations with an impact on general health and well being as well as health maintenance in both dogs and cats. At the same time the potential to reduce safety risk related to new probiotic microorganisms in animal health and welfare should be assessed. Special attention should be placed to develop tools to monitor probiotic activity during processing and storage of canine and feline probiotics.

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