Understanding the canine intestinal microbiota and its modification by pro-, pre- and synbiotics – what is the evidence?

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Review

Understanding the canine intestinal microbiota and its modification by pro-, pre- and synbiotics – what is the evidence?

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Abstract

Interest in the composition of the intestinal microbiota and possibilities of its therapeutic modifications has soared over the last decade and more detailed knowledge specific to the canine microbiota at different mucosal sites including the gut is available. Probiotics, prebiotics or their combination (synbiotics) are a way of modifying the intestinal microbiota and exert effects on the host immune response. Probiotics are proposed to exert their beneficial effects through various pathways, for example production of antimicrobial peptides, enhancing growth of favourable endogenous microorganisms, competition for epithelial colonisation sites and immunemodulatory functions. Despite widespread use of pro-, pre- and synbiotics, scientific evidence of their beneficial effects in different conditions of the dog is scarce. Specific effects of different strains, their combination or their potential side-effects have not been evaluated sufficiently. In some instances, *in vitro* results have been promising, but could not be transferred consistently into *in vivo* situations. Specific canine gastrointestinal (GI) diseases or conditions where probiotics would be beneficial, their most appropriate dosage and application have not been assessed extensively. This review summarises the current knowledge of the intestinal microbiome composition in the dog and evaluates the evidence for probiotic use in canine GI diseases to date. It wishes to provide veterinarians with evidence-based information on when and why these products could be useful in preventing or treating canine GI conditions. It also outlines knowledge about safety and approval of commercial probiotic products, and the potential use of faecal microbial transplantation, as they are related to the topic of probiotic usage.

Keywords: chronic enteropathy, dog, inflammatory bowel disease, microbiome, probiotic.

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Introduction

Microorganisms are found abundantly in association with mammalian hosts; in fact, the number of microbial cells is around 10 times that of host cells (Gibson & Roberfroid 1995), consisting of around 1000 times more microbial genes (The Human Microbiome Project; www.http://hmpdacc.org). The concept of the microbiome was first suggested by Joshua Lederberg, who coined the term 'microbiome' to 'signify the ecological community of commensal, symbiotic and pathogenic microorganisms that literally share our body space' (Lederberg & McCray 2001). It is generally accepted that the term 'microbiota' (in the past also referred to as microflora) is used to describe bacterial communities on mucosal surfaces (with or without luminal microorganisms) or on other body sites (e.g. skin). Overlapping, but distinct, the term 'microbiome' is nowadays used to refer to the entire genetic mass ('genome') of microorganisms. It is mostly, but incorrectly, used to describe *bacterial* genomic communities. One should probably refer specifically to the 'bacteriome', 'virome'(Mansfield 2015) or 'mycobiome' (Foster *et al.* 2013), respectively, and use the term microbiome globally for all microorganisms combined (ten Oever & Netea 2014). These microorganisms include eucaryotes, archaea, bacteria, viruses and fungi. Living in such

close contact, they usually are not harmful to the host; but considered beneficial in most cases. For example the intestinal microbiota supplies short-chain fatty acids (SCFA) as nutrients for colonocytes (Cummings 1981; Rérat *et al.* 1987; Cummings & Macfarlane 1991).

In recent years, more and more research has focused on characterising microbiota at different body sites in people, leading to the Human Microbiome Project (The NIH HMP Working Group 2009); and considerable progress has also been made in defining and understanding microbial communities in small animals, especially as the availability of large-scale genomic sequencing techniques.

Naturally, the detection of differences in microbiota characteristics or the microbiome composition between healthy and diseased subjects has led to the conclusion that modifying these microbial communities might have a beneficial effect on host health in certain circumstances. This is where the application of pre- or probiotics or their combination (so called synbiotics) has been the focus of much attention; especially in human and canine intestinal diseases like inflammatory bowel disease (IBD) (Sauter *et al.* 2006; Ghouri *et al.* 2014; Rossi *et al.* 2014; Schmitz *et al.* 2014, 2015a; Saez-Lara *et al.* 2015).

As probiotics are not usually defined as drugs, they do not have to undergo any process proving their efficacy in applications, diseases or even target species. Hence, many medical claims have been made regarding their beneficial effects, both in humans and in animals. This review focuses on what is known about the definitions, mechanisms of action and efficacy of probiotics in different GI diseases in dogs, and summarises these findings so that the reader can make a better judgement about when and how to use probiotics in small animal gastroenterology.

Composition of the gastrointestinal microbial communities in dogs

The gastrointestinal microbiome in healthy dogs

Recently, high-throughput DNA sequencing techniques have improved microbial identification in small animals. Mostly, these techniques have been applied to describe the phylogenetic structure and functional capacity of the GI microbiome (Handl *et al.* 2011; Swanson *et al.* 2011; Hooda *et al.* 2012). Some studies have included mucosal samples or intestinal content of different segments of the GI tract (Suchodolski *et al.* 2009, 2010, Suchodolski *et al.* 2012a), but most have focused on analysis of faecal samples, as these are easier to obtain (Garcia-Mazcorro *et al.* 2011; Handl *et al.* 2011, 2013; Swanson *et al.* 2011; Suchodolski *et al.* 2012b; Honneffer *et al.* 2014; Minamoto *et al.* 2014a). This is important when comparing studies with each other, as bacterial populations have been shown to differ between different substrates and intestinal sites (Momozawa *et al.* 2011).

In general, bacterial number and diversity increase gradually along the GI tract (Suchodolski *et al.* 2005) (Fig. 1). In the healthy canine stomach, total bacterial load is comparably low (10^5 log10 16S rRNA copy numbers), and mostly belong to Proteobacteria (99.6% of obtained gene sequences) with only few Firmicutes (0.3%) (Garcia-Mazcorro *et al.* 2012). The predominant species are *Helicobacter* and *Lactobacillus* spp. The healthy canine duodenal microbial community as reported by one study to consist of six primary phyla: Firmicutes (46.4% of obtained 16S rRNA gene sequences), Proteobacteria (26.6%), Bacteroidetes (11.2%), Spirochaetes (10.3%) Fusobacteria (3.6%) and Actinobacteria (1%) (Xenoulis *et al.* 2008). Another study evaluated the microbiota in the jejunum of healthy dogs, and identified Proteobacteria as the most abundant (46%), followed by Firmicutes (15%), Actinobacteria (11.2), Spirochaetes (14.2), Bacteroidetes (6.2%) and Fusobacteria (5.4%) (Suchodolski *et al.* 2009) (Fig. 1). Duodenal and jejunal ingesta samples contained 22% and 10% of Lactobacillales respectively (Xenoulis *et al.* 2008). Suchodolski *et al.* (2009) also identified four additional phyla in the jejunum that were not reported in dogs previously: Tenericutes, Cyanobacteria, Verrucomicrobia and Chloroflex, which were all present in low frequency (<0.1%) (Suchodolski *et al.* 2009). The ileal ingesta from healthy research dogs predominantly contained Fusobacteria, Firmicutes and Bacteroidetes (Suchodolski *et al.* 2008). This is significantly

different to the composition of the duodenal and jejunal microbiome composition, as especially the orders of Fusobacteriales (30%), and Clostridiales (22%) with both Clostridium clusters XI and XIVa were predominant in the healthy ileum. Also in contrast to duodenal and jejunal samples, ileal contents had much lower proportions of *Lactobacillus* spp. (1.4%). Whether these variations are true qualitative and quantitative differences is hard to assess, as some of them – especially between different studies – are likely due to variations in the different DNA extraction methods, differences in amplification primers, and sequencing platforms used (e.g. 454-pyrosequencing vs. 16S rRNA clone libraries). Colon samples showed the co-dominant phyla of Fusobacteria, Bacteroidetes and Firmicutes (around 30% each) in healthy dogs (Suchodolski *et al.* 2008). The presence of Fusobacteria (10^8 cfu/ml of intestinal content) also has been demonstrated using culture-based techniques (Davis *et al.* 1977). The next abundant order present was Clostridiales (18%), with *Clostridum* cluster XIVa being the predominant member (50%) (Suchodolski *et al.* 2008). This cluster includes *Eubacterium*, *Roseburia* and *Ruminococcus* spp., which are dietary fibre fermenters. Proteobacteria, including *E. coli*-like organisms, were present in low proportions (1.4%), whereas Lactobacillales where present at levels similar to the jejunum (10%) (Suchodolski *et al.* 2008). Results of the analyses of the canine faecal microbiome indicated a predominance of the phyla Fusobacteria (24–40%), Bacteroidetes (32–34%), Firmicutes (15–28%), Proteobacteria (5–6%) and Actinobacteria (0.8–1.4%) (Xenoulis *et al.* 2008; Suchodolski *et al.* 2009; Middelbos *et al.* 2010; Handl *et al.* 2011; Swanson *et al.* 2011; Garcia-Mazcorro *et al.* 2012) (Fig. 1).

The effect of dietary intervention on the canine gastrointestinal microbial composition

Even though some studies have provided evidence that the administration of prebiotics/fibre in the diet has the ability to manipulate the GI microbiota of dogs (Spears *et al.* 2005; Beloshapka *et al.* 2013;

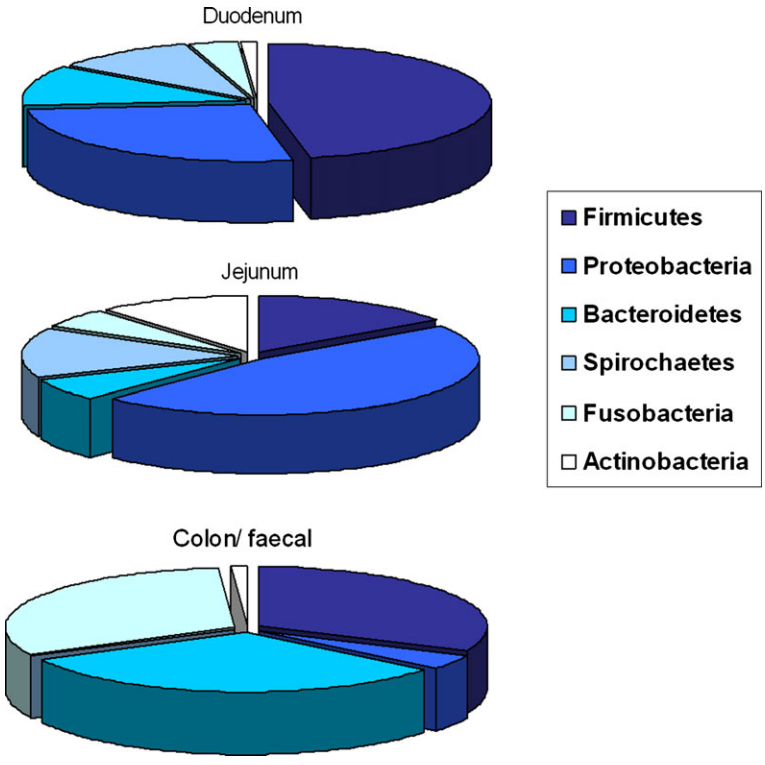

Fig. 1 Distribution of typical bacterial phyla within different compartments of the intestinal tract in dogs.

Panasevich *et al.* 2014), there are some limitations. Firstly, traditional plating techniques or qPCR to quantify a limited number of bacteria (e.g. *Lactobacillus sp., Bifidobacterium sp., Clostridia, E. coli*) were mostly used (Spears *et al.* 2005; Strompfová *et al.* 2012a). Second, most studies were performed in healthy dogs, which make inferring results to diseased dogs or animals susceptible to GI disturbances (e.g. weanlings and geriatrics) difficult. Third, faecal samples were analysed rather than mucosal biopsies or ingesta (Spears *et al.* 2005; Verlinden *et al.* 2006; Hang *et al.* 2012; Strompfová *et al.* 2012b). Lastly, a wide range of prebiotic products and dosages have been administered, making comparisons between studies difficult. The authors of this review recently analysed the faecal microbiome of dogs with food-responsive chronic enteropathy (CE) before and after 6 weeks of dietary intervention and compared these to the faecal microbiome of healthy dogs before and after they were switched to the same diet used in the diseased dogs (a hydrolysed protein diet), and could not detect a significant effect on microbial composition or diversity attributed to the dietary change (Schmitz *et al.*, unpublished data). More research using molecular sequencing techniques is clearly needed to examine the effect of dietary interventions in healthy dogs and dogs with GI disease.

The canine gastrointestinal microbial composition in disease

The invasion and/or colonisation of the GI tract with specific pathogens may profoundly disturb the integrity of the intestinal epithelial barrier (Viswanathan *et al.* 2009). Several potential GI pathogens are recognised in dogs, including *Clostridium perfringens*, *Salmonella* spp. and *E. coli* (Marks *et al.* 2002). However, most of those are also recognised commensals and have been isolated at similar frequencies from dogs with and without signs of GI disease (Marks & Kather 2003; Unterer *et al.* 2014; Busch *et al.* 2015). Therefore, the cause and effect relationship between those organisms and GI disease needs to be interpreted with caution.

Non-specific alterations of the GI microbiota have been regarded as a pivotal factor for the development of acute or chronic GI disease. Several studies have attempted to characterise the faecal microbial composition in diarrhoeic dogs. In acute diarrhoea, large-scale changes were observed, both with culture and sequencing techniques. This included increased abundance of *Clostridium* spp. (especially *C. perfringens*), *E. coli*, *Lactobacillus* and *Enterococcus* spp. with concurrent reductions of those bacterial groups that make up the majority of the normal colonic microbiota, such as *Faecalibacterium,* Ruminococcaceae and *Blautia* spp. (Bell *et al.* 2008; Minamoto *et al.* 2014b; Guard *et al.* 2015). In chronic diarrhoea, significantly higher counts of *Bacteroides* sp. were found using fluorescent in situ hybridisation analysis (Jia *et al.* 2010). In a study evaluating a large group of dogs with chronic diarrhoea using qPCR assays, diseased dogs had significantly decreased abundances of Fusobacteria, Ruminococcaceae, *Blautia* spp. and *Faecalibacterium* spp. and significantly increased abundances of *Bifidobacterium* spp., *Lactobacillus* spp. and *E. coli* compared to healthy dogs (Minamoto *et al.* 2014b).

In samples from dogs with IBD, microbiome changes similar to the ones observed in people with IBD were detected. A significantly reduced species richness and higher proportion of Enterobacteriaceae were observed in duodenal brush samples from dogs with IBD compared with healthy dogs (Xenoulis *et al.* 2008). Additionally, in duodenal mucosal biopsies, a higher abundance of Proteobacteria and lower amount of Clostridia were found in IBD compared with healthy dogs (Suchodolski *et al.* 2010) (Fig. 2). The analysis of faecal samples from dogs with IBD revealed dysbiosis, with significantly lower bacterial diversity, an increase in Gammaproteobacteria (i.e. *E. coli*) and decreases in Erysipelotrichia, Clostridia and Bacteroidia (Minamoto *et al.* 2014a, b).

Whether these changes are partially a cause or a result of the aberrant immune reactions seen in the GI tract in IBD remains a matter of debate, both in people and in dogs. However, it is now suspected that these bacterial changes are associated with altered metabolic functions of the microbiota (e.g. decrease in SCFA concentrations, altered amino acid metabolism, changed in redox equilibrium, altered

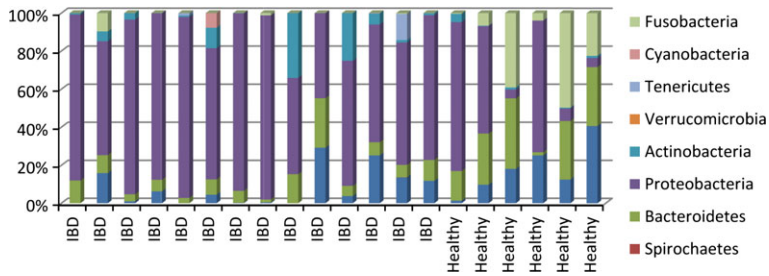

Fig. 2 Distribution of bacterial phyla in the duodenum of 14 dogs with inflammatory bowel disease (IBD) and six healthy dogs (based on: Suchodolski *et al.* 2012a, b).

bile acid metabolism), and are therefore potentially exacerbating the inflammatory state of the host (Hall 2011; Minamoto *et al.* 2014a; Guard *et al.* 2015).

Definition of probiotics, prebiotics and synbiotics

Probiotics are most frequently defined as live microorganisms, which when consumed in adequate amounts confer a health benefit on the host (FAO/WHO, 2002). However, in a lot of circumstances, their health benefits are not strictly proven for a given disease, application or host organism, but the term probiotics is still used. It would be more appropriate to describe probiotics in small animals as live microorganisms given with the intention of improving host health. They include exogenous and indigenous bacterial species that interact with various cellular components within the host (see below).

Prebiotics are defined as selectively fermented ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus also being a benefit to the host organism (Gibson *et al.* 2010; Roberfroid *et al.* 2010). Usually, prebiotics are fibre compounds of different length that pass undigested through the gastrointestinal tract. These include disaccharides (lactulose, tagatose), oligo- or polysaccharides [fructo-oligosaccharides (FOS), mannan oligosaccharides (MOS) xylooligosaccharides, polydextrose, galacto oligosaccharides] or long-chain prebiotics like inulin (Hughes & Rowland 2001; Ogué-Bon *et al.* 2010; Roberfroid *et al.* 2010; Koh *et al.* 2013).

Finally, *synbiotics* are preparations combining both probiotics and prebiotics. This concept was first introduced as 'mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, thus improving host welfare' (Gibson & Roberfroid 1995). The Food and Agriculture Organization of the United Nations (FAO) recommends that the term synbiotic should be used only if the net health benefit observed is synergistic.

Mechanisms of probiotic action

Probiotics can enhance mucosal health by several proposed mechanisms, including displacement of intestinal pathogens (Lee *et al.* 2003), production of antimicrobial substances (Jones & Versalovic 2009), enhancement of immune responses (Pagnini *et al.* 2010), and/or up-regulation of various metabolites (Soo *et al.* 2008).

Probiotics can compete with potential pathogens by interfering with their adherence to the intestinal mucosa or by induction of mucus/mucin production (Collado *et al.* 2007a). These mechanisms are thought to be strain specific, with some strains having increased adherence capabilities (e.g. *L. rhamnosus GG = LGG*), and some strains being able to increase the adherence of pathogens to intestinal mucus (Collado *et al.* 2007b). In addition, probiotic bacteria can produce various antimicrobial substances, for example fatty acids, lactic acid and acetic acid (Saarela *et al.* 2000). Some *Lactobacillus* spp. can decrease toxin gene expression and production by *Salmonella*, *E.coli* or *C. perfringens in vitro* (Medellin-Peña *et al.* 2007; Allaart *et al.* 2011; Bayoumi & Griffiths 2012) or inactivate toxins by

production of proteases *ex vivo* (Castagliuolo *et al.* 1999). Immune modulation of the host organism – especially intestinal epithelial cells (IECs)– might occur through microbial cell wall components, their metabolites or DNA (Oelschlaeger 2010; Thomas & Versalovic 2010). The effects (again mostly shown *in vitro*, but also in some animal models of inflammation) include maintenance and fortification of tight junctions, prolonging the survival of IECs and induction of IgA and β-defensin production (Oelschlaeger 2010; Thomas & Versalovic 2010).

Intact, viable bacteria may be essential for probiotic effects, or these effects could be mediated by a cell wall component or structurally diverse secreted molecules, e.g. peptides, lipopeptides, lipopolysaccharides (LPS), DNA, RNA (Lauková *et al.* 2004). Several mechanistic studies show that key biological signalling pathways like nuclear factor kappa B (NFκB), mitogen-activated protein (MAP) kinases, Akt/phosphatidyl inositol-3 kinase (PI3K) and peroxisome proliferated activator of transcription receptor gamma (PPARγ) are targets for probiotics or their products both *in vitro* and *in vivo* (Thomas & Versalovic 2010) (Fig. 3). These pathways can be modified in different ways by individual probiotic strains. This is clearly a strain-specific effect, as even bacterial strains of the same species can alter cellular responses differentially. For example, *Lactobacillus reuteri* ATCC PTA 6475 can inhibit LPS-induced tumour-necrosis factor alpha (TNFα) production from myeloid cells *in vitro* through suppression of the activator protein-1 (AP-1) pathway, whereas another *L. reuteri* strain, DSM 17938, does not inhibit LPS-induced TNFα production (Lin *et al.* 2009). The details on cellular interactions of specific probiotic strains have been summarised in several

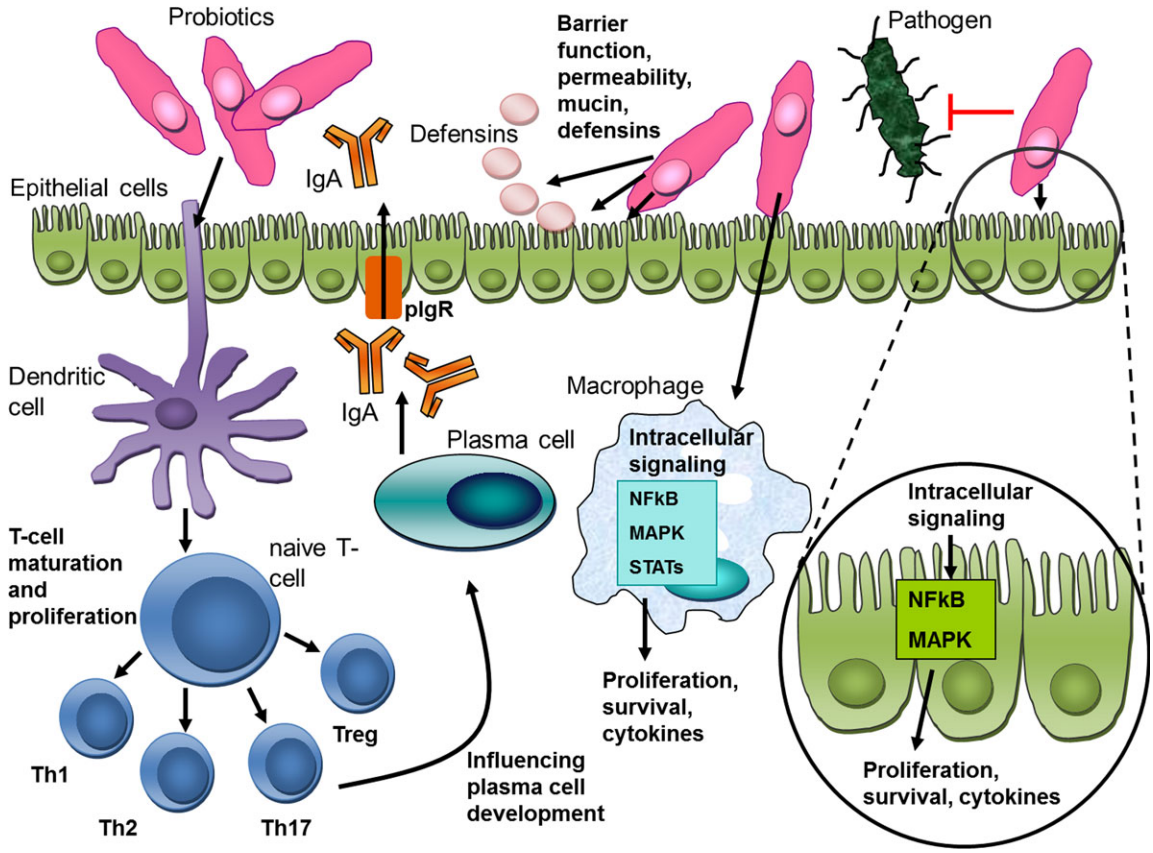

Fig. 3 Proposed mechanisms of action of probiotics (modified from Thomas & Versalovic 2010).

reviews (Oelschlaeger 2010; Thomas & Versalovic 2010; Fijan 2014; Vitetta *et al.* 2014) (figure 3).

Effects of probiotics on intestinal and overall health have mostly been studied in humans and rodent models of human disease (Culligan *et al.* 2009); much more limited data are available for veterinary species (Callaway *et al.* 2008). Although probiotics and prebiotics are administered to dogs with increasing frequency, only few investigations have evaluated the complex interplay of probiotics with small animal host cells, immune function or their effect on the intestinal microbial composition (Garcia-Mazcorro *et al.* 2011). Most of these *in vivo* studies in dogs were rather crude *ex vivo* experiments (Sauter *et al.* 2005; Schmitz *et al.* 2013, 2014). In addition, most investigations specific for companion animals have only studied the effects of selected probiotic strains or probiotic mixtures on the microbiome or other target effects, for example cytokines (Sauter *et al.* 2005; Schmitz *et al.* 2013, 2014).

Microbial organisms commercially used as probiotics in dogs in Europe

To date, four bacterial strains/products have been examined by the European Food Safety Authority (EFSA) for their safety and efficacy as probiotics or feed additives in dogs. This includes two *Enterococcus faecium* strains (*E. faecium* NCIMB 10415 E1705, *E. faecium* NCIMB 10415 E1707), *Lactobacillus acidophilus* DSM 13241 25 and *Bifidobacterium sp. animalis*.

Both products containing an *E. faecium* strain had already been approved for the use in farm animals at the time approval for small animals was sought (2004). For one of these strains, EFSA's conclusion was that enough information was provided to consider it safe for the use in dogs and for humans having contact with treated dogs (*E. faecium* NCIMB 10415 E1707). The other *E. faecium*, strain NCIMB 10415 E1705, was considered unlikely to represent a hazard for the target species even when supplied in overdose. It was shown to not favour the growth and shedding of haemolytic and non-haemolytic *E.coli* in dogs (and cats).

For the product containing *Lactobacillus acidophilus* DSM 13241 25, EFSA did not establish a safety concern, as the strain was sensitive to medically relevant antibiotics, with the exception of ciprofloxacin. As no data on the effect of this probiotic on shedding of intestinal pathogens in the dog were provided, and it was considered a potential respiratory sensitiser, further data were requested by EFSA before reaching a final conclusion (2004).

The latest probiotic strain assessed was *Bifidobacterium animalis* (2012). For this strain (no further strain designation or details are available), the requirements regarding the assessment of antibiotic resistances were not met (as the strain was resistant to clindamycin and the genetic basis of the resistance could not be established). Based on two studies provided, the effect of *B. animalis* on GI-related parameters in dogs was considered of questionable biological relevance and EFSA could not conclude the efficacy of this product.

Apart from the strains mentioned above, other probiotics or synbiotics are available as nutritional supplements in dogs, both in Europe and in the USA. Even though most products available in Europe to date contain the *E. faecium* strain NCIMB 10415 E1707, sometimes in combination with other bacterial strains and different prebiotics, the products themselves have mostly not been specifically approved or tested. On the other hand, *E. faecium* NCIMB 10415 E1707 has been used most widely in experimental settings, to assess effect on immune function or gut health (see below). Other bacterial strains available as over-the-counter supplements for dogs contain different strains of *Lactobacilli* (*L. acidophilus, L. casei, L. plantarum, L. paracasei, L. lactis, L. rhamnosus, L. salivarius*), *Bifidobacteria* (*B. infantis, B. lactis, B. longum, B. bifidum*), *Bacillus subtilis* or *coagulans* and in some cases yeasts (*Saccharomyces cerevisiae*) or other fungi (*Aspergillus oryzae*). However, limited data are available about the safety and efficacy of these microorganisms/products or the health claims associated with them. Some microorganisms other than *E. faecium* have been tested as probiotics in experimental settings in dogs. For example *Saccharomyces boulardii* was investigated in a small pilot study presented as a congress

abstract in dogs with IBD and protein-losing enteropathy (P-LE); (Bresciani *et al.* 2014)]. It significantly improved clinical activity score and serum albumin levels compared to the placebo-treated control dogs (Bresciani *et al.* 2014). Apart from single bacterial strains tested *in vitro* (detailed below), some single- and multi-strain probiotic products have also been tested to a certain degree in a clinical setting in dogs. For most single-strain studies, this is limited to the use of *E. faecium* (Swanson *et al.* 2002; Sauter *et al.* 2006; Strompfová *et al.* 2006; Schmitz *et al.* 2015a). Several probiotic cocktails have been used with variable effect. For example a mixture of lactobacilli that showed promising *ex vivo* results regarding creating a more tolerant microenvironment in the gut, did not significantly improve outcome when administered in a clinical trial (Sauter *et al.* 2006). In another study, strains from a product approved for the use in people (VSL#3) have been administered to dogs with IBD leading to some clinical and immunological improvement (Rossi *et al.* 2014). This includes four *Lactobacilli* (*L. acidophilus, L. plantarum, L. paracasei, L. delbrueckii ssp. bulgaricus*), three *Bifidobacteria* (*B. breve, B. longum, B. infantis*) and *Streptococcus thermophilus*.

Testing of microbial strains for their qualifications as probiotics in dogs

Several bacterial strains, mostly lactic acid producing bacteria (LAB) isolated from canine faeces, some originally used in other species or people, have been tested for their probiotic potential *in vitro* (but are currently not available in commercial products). Studies have especially focused on the survival properties of these strains at low pH (to mimic passage of the stomach), to resist degradation by bile acids in the small intestine, their adhesion properties to intestinal mucus and their potential to either produce antimicrobial peptides or to inhibit *in vitro* growth of pathogens (mostly *E. coli* and *Salmonella* ssp.). Some other functional and genetic properties (e.g. fermentation of carbohydrates, immune-modulating effects) have also been assessed. These studies and their main outcome are summarised in Table 1.

Very little is known about the appropriate dose of probiotics in general in small animals, let alone in specific diseases. Survival characteristics of probiotic strains (especially *E. faecium*) have been tested *in vitro* and *in vivo*, that is in low environmental pH (mimicking gastric passage), the presence of bile, adhesion to mucus, recovery of live bacteria from faeces of dogs after oral administration (Lauková *et al.* 2004, 2008; Strompfová *et al.* 2004a; Marciňáková *et al.* 2006). Overall, it is not entirely clear if probiotic survival is even necessary for a beneficial effect, or if, for example their DNA is sufficient (Kant *et al.* 2014). There is some evidence that even non-viable probiotic bacteria can cause immune modulation in the host (Zhong *et al.* 2012). Also, there is very little information regarding possible interactions of strains in multi-strain formulations; even though some studies in experimental rodents or humans show a synergistic effect on the measured outcome (Baillon *et al.* 2004). The effect of formulation (liquid, capsule, in-feed) or natural sources of potential probiotics (yoghurt, raw green tripe, fermented plant material) is mostly unexplored to date. Some preliminary data on the effect of feeding raw meat, prebiotic fibre and yeast cell wall extract on the composition of faecal microbiota are available (Beloshapka *et al.* 2013). Changes depending on the meat protein source (chicken vs. beef) were less evident, but minor changes in faecal microbiota composition were seen when prebiotics were added (e.g. greater presence of fusobacteria, lower abundance of *Faecalibacterium*). The significance of these observed changes, however, remains unclear, especially as this study was performed in healthy dogs. Furthermore, most studies evaluated faecal samples and noticed only minor changes. However, recent studies suggest that probiotic mixtures (i.e. VSL#3) are able to induce major changes in the ileal mucosa-adherent microbiota in colitic mice and also dogs with chronic enteropathies (Mar *et al.* 2014; White *et al.* 2015).

Quality control is also an issue with probiotic products. As they are usually classified as nutritional supplements, quality control as for drugs is not legally required. Again, no large amount of data are available on the quality, shelf-life, etc., of commercially available probiotics. One study has assessed

Table 1. List of bacterial strains isolated from canine substrates tested *ex vivo* for their probiotic properties

Bacterial strain(s)	Source	Tested for	Result	Reference
Butyrivibrio fibrisolvens	Canine faeces	Presence and butyrate production	*B. fibrisolvens* was detected at low levels (2.4×10^3–9×10^5/g dry weight) in canine faeces and was only responsible for at most 30% of the butyrate production by mixed faecal microbes. Introduction of *B. fibrisolvens* at high number might increase butyrate and lactate production	Asanuma *et al.* (2001)
L. fermentum, L. mucosae, L. rhamnosus, L. salivarius, Weissella confusa	Canine faeces	MIC of 14 antibiotics, viability at pH2 for 4 h, antimicrobial activity against *Micrococcus luteus*	*L. salivarius* and *W. confusa* are possible probiotics	Beasley *et al.* (2006)
16 isolates of *Bifidobacterium animalis* ssp. *lactis*	Canine faeces (GSD)	Survival in an in vitro digestion assay, viability at low pH, viability with bile salts, auto-aggregation activity	All strains showed good properties as potential probiotics	Bunešová *et al.* (2012)
L. fermentum, L. plantarum, L. rhamnosus and their combination	Unclear, but claimed to be 'dog-specific'	Adhesion to canine mucus, effect of different growth media (laboratory vs. manufacturing conditions), inactivation methods (80°C, 95°C and UV irradiation)	Better adhesion under laboratory conditions, inactivation by heat decreased adhesion properties	Grześkowiak *et al.* (2013)
Lactobacilli, Bifidobacteria and *Enterococci*	Canine large intestinal contents	Isolation frequency, similarity to known gene sequences	LAB are frequently found in canine faeces: 78% *Lactobacillus*, 11.6% *Enterococcus*, 6.8% *Bifidobacterium*, 2% *Streptococcus bovis*	Kim & Adachi (2007)
Enterococci	Dog faeces and dog feed	Adhesion to canine, porcine and human mucus	*E. faecium* and *E. faecalis* exhibit strain-dependent *in vitro* adhesion to human, canine and porcine mucus with no host specificity	Lauková *et al.* (2008)
Enterococci	28 different commercially available dog feeds	Species identification, antibiotic sensitivity profiles, adhesion to human and canine mucus, lactic acid production, viability in bile	22 selected strains classified: 6 *E. faecium*, 4 *E. faecalis*, 1 *E. hirae*, the remainder not classified. Good qualities as probiotics with no significant antibiotic resistance	Lauková *et al.* (2004)
Lactobacilli	Canine milk	Culture-based characterisation of lactobacilli (fermentation of carbohydrates, production of antimicrobial peptides, adhesion to mucin, MIC to antibiotics)	Some *Lactobacillus* sp. strains showed potential as probiotics	Martín *et al.* (2010)
Lactobacilli	Canine faeces	Bile resistance, inhibition of *Salmonella typhimurium*, production of reuterin	*L. reuteri* was dominant species, was more bile resistant, produced reuterin, inhibited *Salmonella* better than *L. acidophilus*	McCoy & Gilliland (2007)
Lactobacilli, Bifidobacteria	Canine intestinal mucosa-adherent microbiota (post-mortem)	pH sensitivity, bile resistance, pathogen inhibition, adherence to epithelial cells, survival after freeze-drying, feeding trial of B. animalis AHC7	B. animals AHC7 showed best properties out of 62 strains of LAB isolated. It also reduced the carriage of *Clostridium difficile* in dogs	O'Mahony *et al.* (2009)
Lactobacilli	Canine faeces	pH sensitivity, bile resistance, inhibition of *E.coli* and *Clostridium perfringens* growth	Some isolates could colonise and persist in the GI tract and induce beneficial effects to the host	Perelmuter *et al.* (2008)

Table 1. Continued

Bacterial strain(s)	Source	Tested for	Result	Reference
Lactobacilli	Canine faeces	*In vivo* administration of *Lactobacillus murinus*	*L. murinus* transiently persisted in the canine gut and is safe for administration	Perelmuter *et al.* (2011)
Lactobacillus acidophilus, Candida utilis enriched with selenium and zinc	Unknown	Blood selenium and zinc concentrations, blood antioxidant capacities, composition of intestinal microflora	Probiotic group: Increased blood selenium and zinc concentrations, increased activity of glutathione peroxidise, superoxide dismutase, total antioxidant capacity, increased *Lactobacillus* and *Bifidobacterium* count in the faeces	Ren *et al.* (2011)
L. rhamnosus GG, L. johnsonii, L. casei, L. bulgaris, L. pentosus	Bacterial strains: unknown, mucus: different species including dog	Adhesion of different probiotic LAB to different host mucus	Mucus adhesion properties are more dependent on the LAB strain than on the host	Rinkinen *et al.* (2003a)
LAB (*L. rhamnosus GG, L. pentosus, B. lactis, E. faecium*)	Unknown, some 'canine' origin	Inhibition of adhesion of canine and zoonotic pathogens (*S. intermedius, S. typhimurium, C. perfringens, C. jejuni*) to immobilised mucus from canine jejunal chyme	LAB of canine origin reduced adhesion of *C. perfringens*. No strain inhibited adhesion of *S. typhimurium* or *S. intermedius*. Both Enterococci enhanced adhesion of *C. jejuni*	Rinkinen *et al.* (2003b)
L. acidophilus (2 strains), *L. johnsonii* as a probiotic cocktail (PC)	Faecal samples from healthy dogs	PH-resistance, fermentation ability, anti-ETEC-activity, lactic acid/peroxide production, antibiotic resistance, storage capability, cytokine expression in canine PBMCs, cytokine expression in canine intestinal biopsies	PC increased IL-10 mRNA levels in healthy and inflamed tissues, especially in comparison to not altering TNFα, IFNγ and IL-12p40 levels → induction of a more tolerant micro-environment	Sauter *et al.* (2005)
Enterococcus faecium NCIMB 10415 E1707	Commercial product	Cytokine production in canine intestinal biopsies and whole blood when co-cultured with EF or other TLR-ligands	Variety of changes in cytokine expression profile dependent on stimulant, TNFα increased in whole blood but reduced in biopsies, single TLR-ligands more potent and consistent than *E. faecium* stimulation	Schmitz *et al.* (2014)
Enterococcus faecium NCIMB 10415 E1707	Commercial product	TNFα responses in whole blood compared to PBMCs from healthy dogs	*E. faecium* consistently produces TNFα responses, generally stronger response in whole blood than in PBMCs	Schmitz *et al.* (2013)
LAB	Faeces of 22 dogs	Characterisation of LAB (*Bifidobacteria* and *Lactobacilli*) in canine faeces (enzymatic activities, resistance to bile, antimicrobial susceptibility, inhibition of 15 indicator bacteria)	*L. murinus, B. animalis* and *Pediococcus acidilactici* most frequently isolated. All strains inhibited Gram-negative indicators (lactobacilli > bifidobacteria). *L. reuteri* showed best antimicrobial properties, resistance to bile observed in all strains, strain differences in pH-stability	Strompfová & Lauková (2014)
Lactobacilli, Enterococci	Faeces from 10 healthy dogs	Antimicrobial activity, tolerance to bile, adhesion properties	40 strains of enterococci and 40 strains of lactobacilli isolated and tested, some showing potential as probiotics	Strompfová *et al.* (2004a)
Enterococci	Canine faeces	Bacteriocin production, pH and bile tolerance, antibiotic resistance, adhesion properties	Total count of 3.3–7.3 log10 CFU g^{-1} faeces, most strains were *E. faecium*, all sensitive to vancomycin, ampicillin, penicillin, chloramphenicol. 33% resistant to erythromycin, 28% to teteracycline. 75% showed broad inhibitor spectrum against Gram-positive indicator bacteria. Seven strains tested further: best probiotic properties: *E. faecalis* EE4 and *E. faecium* EF01	Strompfová *et al.* (2004b)

Table 1. Continued

Bacterial strain(s)	Source	Tested for	Result	Reference
Lactobacillus acidophilus	Canine jejunal chyme	Detection of specific *L. acidophilus* LAB20 strain using real-time PCR	LAB20 can be detected from canine faecal samples up to 6 weeks post-administration, could be candidate to study mechanism behind its persistence in the canine gut, could be probiotic candidate	Tang & Saris (2013)
Lactobacilli	Canine faeces	Effect of different carbon sources on the production of antimicrobial compounds against *E.coli* and *S. typhimurium* by 3 lactobacilli strains	Substrate affects the production of antimicrobial compounds by *L. mucosae*, *L. acidophilus* and *L. reuteri* in a dose- and pH-dependent manner	Tzortzis *et al.* (2004)
LAB	Canine faeces	Oxalate degradation by LABs, effect of different prebiotics (arabinogalactan, gum Arabic, lactitol, guar gum, inuin, maltodextrin, FOS) on oxalate degradation	37 LAB were isolated, mean oxalate degradation was 17.7 ± 16.6%. The effect of prebiotics was variable, but overall greatest with guar gum. Manipulation of LAB might decrease intestinal oxalate, thus potentially reducing oxalate absorption and urolithiasis risk	Weese *et al.* (2004)

microbial components of dog food claiming to contain probiotics (Weese & Arroyo 2003). None of the 19 commercial diets contained all the claimed organisms, whereas one or more of the listed components could be isolated from around 50% of samples. Eleven samples contained additional, related organisms and more than 25% of tested diets showed no relevant bacterial growth. To the authors' knowledge, no published study has so far assessed whether the claimed bacterial quality or quantity in probiotic nutritional supplements is according to label claims.

The effect of probiotics on selected parameters in healthy dogs

Enterococcus faecium

Interestingly, even though *E. faecium* is the most widely used probiotic strain in small animals, not many studies have focused on its safety or effects when administered to healthy dogs. In a study from 2003 performed at the Nestle Purina Product Technology Centre, puppies from different popular dog breeds were assigned one of two different diets after weaning (Benyacoub *et al.* 2003). One of the diets was a commercial, complete dog food with no supplements (control group), the other was the same diet with a stable encapsulated form of *E. faecium* 10415 SF68 in a dosage of 5×10^8 cfu day^{-1} added (treatment group). Main outcome measures included determination of total faecal IgA, total and vaccine-specific immunoglobulin gamma (IgG) and IgA serum concentrations, and quantification of circulating lymphocyte subsets by flow cytometry. Food intake, weight and routine laboratory parameters (complete blood count, serum biochemistry) were also evaluated. At the end of the study period (1 year), puppies consuming the test diet had significantly higher total faecal and serum IgA levels (but not serum IgG) compared to the control group. In addition, vaccination-associated IgA and IgG for canine distemper virus were also significantly higher in *E. faecium*-treated puppies compared to controls from week 31 on. All other parameters were not different between groups. This study concluded that *E. faecium* can enhance specific immune functions in

young dogs (Benyacoub *et al.* 2003), but the clinical relevance is questionable as measured endpoints do not necessarily correlate to a 'healthier' intestine. Overall, evidence justifying the use of *E. faecium* under these conditions is limited.

In the second study investigating effects of *E. faecium* on healthy dogs, the strain used (*E. faecium* EE3) was isolated and enumerated from a commercially available canine food (Marciňáková *et al.* 2006). It was orally administered to healthy adult dogs of different breeds and ages for 1 week at a daily dosage of 2–3 $\times$ 10^9 cfu dog^{-1}. Faecal cultures and blood samples for routine biochemistry values were obtained before and at the end of the treatment period, as well as 1, 2 and 3 months after cessation of probiotic administration. *E. faecium* treatment did not cause any clinical side-effects. The strain persisted in faeces for 3 months after cessation of treatment (reaching average concentrations of 6.83 $\pm$ 0.95 log cfu g^{-1}). Total concentration of LAB increased, and *Pseudomonas*-like bacteria and *Staphylococcus* spp. decreased in faecal samples, but the abundance of *E. coli* was not influenced. Total serum lipids and protein decreased in most dogs with treatment, with cholesterol being within the reference range of all dogs at the end of the treatment period. This study, therefore, inferred a beneficial influence of *E. faecium* on canine health, possibly even in obesity, even though obese animals were not examined in the study, and the beneficial effect of normalisation of cholesterol is questionable (Marciňáková *et al.* 2006). In addition, this is the only study that has shown long-term persistence of orally administered probiotics in dogs, there was a lack of a control group and it is not entirely clear how the strain identification was performed; hence the results of this study have to be interpreted with caution. Also, for both of these studies, it has to be questioned if the definition of probiotics has been fulfilled using *E. faecium*, as a relevant benefit of increased faecal IgA, elevated vaccine-associated titres or 'lowered' cholesterol has not been demonstrated.

Lactobacilli and bifidobacteria

A number of studies have investigated the safety and effect of single-strain LAB preparations in healthy dogs. For example effects on measured outcomes of the administration of *L. acidophilus* have been controversial. In one study, its administration (*L. acidophilus* DSM13241) to adult dogs was associated with changes in haematological and immunological parameters (increased red blood cells [RBC], Hct, haemoglobin, neutrophils, monocytes and serum IgG, reduced RBC fragility and serum NO concentrations) of questionable clinical relevance (Baillon *et al.* 2004). In another study, the supposedly beneficial changes observed in the composition of the microbiota and faecal metabolites was more attributed to the addition of prebiotic FOS than to *L. acidophilus* NCFM itself (Swanson *et al.* 2002).

L. fermentum has been tested by the same group in two studies (Strompfová *et al.* 2006, 2012a). This LAB (*L. fermentum* AD1) was originally isolated from faeces of a healthy dog (6-year-old Tibetan Terrier) and was shown to have good *in vitro* survival at a pH of 3.0 for 3 h (86.8%), and in the presence of 1% bile (75.4%), as well as good adhesion properties to canine and human intestinal mucus, and no unacceptable antimicrobial resistance (Strompfová *et al.* 2006). It was orally administered to 15 healthy dogs of various breeds at a dose of 3 $\times$ 10^9 cfu dog^{-1} for 7 days. Significant increases of faecal lactobacilli, enterococci, and serum total protein, total lipids and reduction in blood glucose were noted. In a follow-up study, the strain was administered in freeze-dried form to healthy dogs, and was shown to persist short-term in the GI tract and to increase SCFA concentrations. Additionally, a reduction in Clostridia and Gram-negative bacteria (coliforms, *Aeromonas, Pseudomonas*) were also noted by faecal culture (Strompfová *et al.* 2012a). In the study, this was implied as a desired outcome, however, as this is a culture-based approach and some members of Clostridia have been identified as part of the normal beneficial gut flora (see above), deductions regarding gut health are difficult to make from these data. More detailed investigations to evaluate whether this probiotic strain reduces specifically the potential pathogen *C. perfringens* rather than Clostridia in general would be needed.

Lactobacillus animalis LA4 (isolated from the faeces of a healthy adult dog) was tested *in vitro* and

in vivo in nine dogs (freeze-dried, given for 10 days). On day 11, cultured faecal lactobacilli concentrations were increased and enterococci reduced compared to the beginning of the trial, hence this strain was concluded to have some probiotic properties (Biagi *et al.* 2007). However, this conclusion is nearly impossible to make, as semi-quantitative culture was the only outcome assessed and there was no control group.

A more recent study used a genetically engineered strain of *Lactobacillus casei* (no further strain designation available), capable of producing biologically active canine granulocyte macrophage colony stimulating factor, and investigated its properties as a probiotic for dogs (Chung *et al.* 2009). It was administered at 1×10^9 cfu day^{-1} for 7 weeks. Treated dogs showed increased monocyte counts, serum IgA and canine coronavirus-specific vaccination-associated IgG compared with dogs fed a regular diet without probiotic supplements and dogs receiving a non-engineered strain of *L. casei* (Chung *et al.* 2009). The clinical relevance of this finding and the safety and efficacy of administration of this strain remains open.

Different strains of *Bifidobacterium animalis* [AHC7; (Kelley *et al.* 2010); and an unspecified strain isolated from dog faeces (Strompfová & Lauková 2014)], were investigated by the same group and found to not cause any undesirable effects. Their administration to healthy dogs increased the number of cultured LAB, but lowered the count of coliform bacteria in canine faeces. Other faecal and serum parameters as well as the phagocytic activity of peripheral blood leucocytes (especially neutrophils) were improved in treated dogs compared with the untreated control group. These effects could be detected several weeks after the treatment had been ceased (Strompfová *et al.* 2014), but again their relevance remains unclear, especially as methods used were rather crude assessments of immune function.

Overall data on improving gut health or immunological status in dogs using lactobacilli or bifidobacteria are not compelling, especially in the light of the fact that it is not known if increasing certain bacterial phyla is correlated with improved GI function or a lower incidence of diarrhoeic diseases.

Bacilli

Bacilli are thought by some authors to represent superior probiotics to LABs, as they can sporulate and thus be more resistant to environmental stress and low pH (Biourge *et al.* 1998; Félix *et al.* 2010). However, mere survival might be not the most important feature of a probiotic, and they all need to be tested for their benefit in clinical situations. In some European countries, probiotic products containing Bacilli are available as nutritional supplements for humans and animals (Biourge *et al.* 1998). They have been shown to have beneficial effects on the survival of mice infected with *Klebsiella pneumoniae* and on the breeding performances of a number of production animals (Biourge *et al.* 1998). *Bacillus* CIP 5832 was found to be persistent when added to canine food and when exposed to expansion-extrusion and drying experiments. They were also able to survive the canine GI tract, however, did not seem to persist, as they disappeared from faeces 3 days after cessation of administration (German *et al.* 2000). Only one study found that *Bacillus subtilis* C-3102 can improve faecal texture and odour in dogs due to a decreased content of faecal ammonia (Félix *et al.* 2010). Once again, the clinical relevance of this finding and the justification of calling bacilli 'probiotics' in this situation is highly questionable and the usage of Bacilli as probiotics cannot be recommended.

Probiotic mixtures

Mixtures of probiotics used in healthy dogs have been of variable composition; in addition, outcome measures were different, mainly also due to the availability of different techniques to assess changes in microbial communities. One study administered five potentially probiotic LAB strains (*L. fermentum, L. salivarius, Weissella confuse, L. rhamnosus* and *L. mucosae*) to five permanently fistulated Beagle dogs for 7 days (Manninen *et al.* 2006). Denaturing gradient gel electrophoresis (DGGE) demonstrated that the LAB modified the dominant indigenous jejunal LAB microbiota. All strains were undetectable 7 days after administration ceased and effects were transient. In another study (Garcia-Mazcorro *et al.*

2011), seven strains of LAB (*E. faecium*, *S. salivarus* ssp. *thermophilus*, *B. longum*, *L. acidophilus*, *L. casei* ssp. *rhamnosus*, *L. plantarum*, *L. delbrueckii* ssp. *bulgaricus*) were administered to 12 healthy dogs and faecal microbial communities were assessed using DGGE gels, 16S rRNA gene libraries, quantitative PCR and 16S rRNA gene 454-pyrosequencing. Probiotic species were detectable in 11/12 dogs during product administration, but not before or after. Abundances of *Enterococcus* and *Streptococcus* spp. were significantly increased. However, on pyrosequencing, no changes in the major bacterial phyla were observed. This study concluded that the product was well tolerated and did not cause any clinical side-effects. Administration of the product resulted in increased abundance of the probiotic genera, but this was not sufficient to cause significant changes in the overall microbiome structure (Garcia-Mazcorro *et al.* 2011) and certainly cannot automatically be inferred to convey a health benefit.

Finally, a synbiotic consisting of *E. faecium* SF68, *Bacillus coagulans*, *L. acidophilus* and several prebiotics (FOS, MOS) and vitamins (B3, B6) was administered in a placebo-controlled trial to healthy trained sled dogs, and changes of the composition of the faecal microbiota assessed using quantitative PCR and tag-encoded FLX 16S rDNA amplicon pyrosequencing (Gagné *et al.* 2013). Alterations in the faecal microbiome observed included a rise in Lactobacillaceae and an increased faecal butyrate concentration across all dogs. Faecal scores also improved compared with the control group at 5 weeks (Gagné *et al.* 2013). Whether these findings are correlated, beneficial to the host or even only incidental, remains unclear.

Use of probiotics in small animal gastrointestinal diseases

Infectious and non-infectious acute diarrhoea

Overall, it seems that – possibly depending on the probiotic strain or mixture used – there is some merit in the use of probiotics in acute infectious canine GI diseases. Administration of the probiotic mixture VSL#3 in a randomised manner to puppies with confirmed parvoviral enteritis lead to an increased percentage of surviving dogs (90% in probiotic group vs. 70% in the non-probiotic group), and a more rapid improvement of clinical scores and leucocyte/lymphocyte counts (Arslan *et al.* 2012). In another study, there was a significant reduction in Ancylostoma eggs shedding in 10 dogs treated with a mixture of LABs (*L. acidophilus* ATCC 4536, *L. plantarum* ATCC 8014 and *L. delbrueckii* UFV H2B20) for 28 days compared with an untreated control group (Coêlho *et al.* 2013). Similar results could not be achieved for the treatment of canine giardiasis with *E. faecium* SF68: after 6 weeks of treatment no differences in cyst shedding, faecal antigen shedding, faecal IgA or leucocyte phagocytic activity were observed between treated and untreated dogs (Simpson *et al.* 2009).

Other forms of acute diarrhoea in dogs in which probiotics have been administered include stress-associated (e.g. kennelling stress), antibiotic-induced and idiopathic diarrhoea. Results are variable, depending on the probiotic strain and the dog population evaluated. Using *E. faecium* SF68, there was no effect on kennel stress-associated diarrhoea, which might partially be due to the low prevalence of diarrhoea in this study (Bybee *et al.* 2011). Faecal scores were significantly improved in dogs undergoing kennelling stress when supplemented with *Bifidobacterium animalis* AHC7 compared with an untreated control group (Kelley *et al.* 2012). The same strain was able to significantly reduce the time to resolution of clinical signs and the number of dogs receiving metronidazole in a study of acute idiopathic diarrhoea (dose of 2×10^{10} cfu day^{-1}) (Kelley *et al.* 2009). Similar responses were seen in a study of acute gastroenteritis using a probiotic mixture of *L. acidophilus*, *Pediococcus acidilactici*, *B. subtilis, B. licheniformis* and *L. farciminis* (Herstad *et al.* 2010). Recovery time was significantly reduced (mean 1.3 days, 95% CI: 0.5–2.1 days) compared with untreated controls (mean 2.2 days, 95% CI: 1.3–3.1 days) with a comparably large dose of 4.2×10^9 cfu/10 kg three times daily (Herstad *et al.* 2010). Inactivated bacterial compounds as part of an 'enterovaccine' might also be useful in treating recurrent self-limiting episodes of diarrhoea (e.g. stress-

related) in dogs. One commercially available preparation (deactivated whole bacteria and lysates of *E.coli*, *Bacillus pumilus*, *Morganella morganii*, *Alcaligenes faecalis*, *Shigella flexneri*, *Bacillus subtilis*, *Enterococcus faecalis* and *Proteus vulgaris*) reduced the number of diarrhoea episodes and severity in five of six treated dogs (Cerquetella *et al.* 2012). There were no control dogs in this pilot study, hence the exact potential of this inactivated bacterial mixture needs to be evaluated by further clinical studies. Interestingly, administration of *Saccharomyces boulardii* to dogs with experimental lincomycin-induced diarrhoea could prevent, but not treat this condition (Aktas *et al.* 2004).

Chronic diarrhoea

Similar to people, the pathogenesis of chronic inflammatory conditions of the GI tract in dogs (e.g. CE/IBD) is assumed to be due to an aberrant response of the immune-system to the luminal or adherent intestinal microbiota (Sartor 2006; Hall & German 2010). There is ample evidence of immune dysregulation, even though the exact type of inflammatory response and pathogenesis has not been elucidated yet (German *et al.* 2000; Peters *et al.* 2005; Jergens *et al.* 2009; Schmitz *et al.* 2012). Several studies have shown that there are also alterations of the intestinal microbiome present in canine CE/IBD (Suchodolski *et al.* 2012a, b). Hence, there have been several attempts to influence the composition of the microbiota in those dogs to alleviate clinical signs; partially using probiotics that had already shown to have some immune-modulatory properties in *in vitro* or *ex vivo* studies (Sauter *et al.* 2005, 2006; Schmitz *et al.* 2013, 2014). *E. faecium* NCIMB 10415 E1707 has been assessed as a single-strain treatment in dogs with food-responsive disease (FRD) and found to have no effect on clinical activity score, histology scores or duodenal and colonic gene expression of selected genes associated with specific T-helper lymphocyte lines (Schmitz *et al.* 2015b). In addition, there was also no effect of *E. faecium* treatment on gene or protein expression of inflammasome compounds (Schmitz *et al.* 2015b). More promising results could be achieved by using probiotic mixtures in FRD dogs: A combination of LAB (*L. acidophilus* and *L. johnsonii*) reduced duodenal interleukin (IL)-10 and colonic interferon gamma (IFNγ) mRNA levels and the number of faecal Enterobacteriaceae, whereas numbers of *Lactobacillus* spp. increased. Clinical improvement was noted to similar levels in dogs receiving the LAB cocktail compared with dogs treated with diet alone (Sauter *et al.* 2005).

Additionally, a probiotic mixture with VSL#3 strains formulated for pets (SIVOY™; details above) was used in dogs with idiopathic IBD and compared to a treatment regimen with metronidazole and prednisolone in an open-label trial (Rossi *et al.* 2014). Clinical activity, duodenal histology scores and $CD3^+$ lymphocytes in the intestinal tissue decreased post-treatment in both groups. However, $FoxP3^+$ cells (a marker of regulatory T-helper lymphocytes [Tregs]) increased significantly after treatment only in the dogs treated with VSL#3 strains. Also, transforming growth factor beta $(TGFb)^+$ cells (most likely Tregs) increased in both groups after treatment, but to a greater magnitude in probiotic treated dogs. There was some effect of the probiotics on expression of tight junction proteins, with occludin being significantly elevated in healthy control dogs and dogs treated with probiotics compared with IBD dogs. Microbiome analysis based on quantitative PCR revealed a reduced abundance of *Faecalibacterium* and *Turicibacter* in dogs with IBD at the start of the trial, with a significant increase in *Faecalibacterium* observed in the animals treated with VSL#3 strains (Rossi *et al.* 2014). This is noteworthy, as *Faecalibacterium prausnitzii* has been advocated as an anti-inflammatory commensal bacterium in people (Miquel *et al.* 2013) and is of lower abundance in intestinal contents or faecal samples from human and canine IBD patients (Suchodolski *et al.* 2012b; Fujimoto *et al.* 2013).

Saccharomyces boulardii yeasts have been administered to dogs with CE/PLE and healthy control dogs in the small pilot placebo-controlled double-blinded clinical trial mentioned above at 1×10^9 cfu kg^{-1} body weight BID for 10 days (Bresciani *et al.* 2014). Dogs with CE or PLE additionally received standard medical treatment consisting of diet, antibiotics and/or immunosuppressive

drugs. *S. boulardii* was not detected in faecal samples of healthy dogs before treatment started, but was present after 1 day of supplementation, reached highest levels after 5 days (10×10^7 cfu g^{-1}) and was eliminated 4 days after withdrawal of treatment. In dogs with CE, clinical score improved significantly, and in dogs with PLE serum albumin values increased significantly compared with placebo treatment. Duodenal endoscopic and histology scores were not different before and after treatment in any of the dogs. The study concluded that *S. boulardii* can be safely administered to dogs and it might be useful as an adjunctive treatment in CE and PLE (Bresciani *et al.* 2014). However, as it is a small study and has not been fully published yet, awaiting of the full results and further research into the usefulness of *S. boulardii* as a potential probiotic is warranted.

Faecal microbial transplants

Administration of single-strain or even multi-strain probiotic products might have a limited ability to influence the composition of the intestinal microbiome permanently, especially given the fact that the microbiome consists of hundreds of microbial species. Based on the assumption that a more complex change is needed in certain conditions (e.g. *Clostridium difficile* infection or IBD), there have been attempts of transferring the intestinal microbiota from one subject to another. This has been termed faecal microbial transplantation (FMT), microbiome restorative therapy or faecal bacteriotherapy. In human medicine, FMT has been mostly performed for recurrent *C. difficile* infection. Two reviews show that it is safe and effective in 83–92% of human patients, which achieved full resolution of clinical signs (Gough *et al.* 2011; Guo *et al.* 2012). Limited clinical data are available evaluating the use of FMT for IBD in humans, but pilot studies suggest that the response rate to FMT in chronic intestinal inflammation is much lower compared with *C. difficile* infection (Colman & Rubin 2014). Experience in small animals regarding the safety and efficacy of FMT is scarce and anecdotal. Two congress abstracts report some preliminary findings in dogs. One is a case report of an ongoing study (not published), where a dog with eosinophilic IBD of 2 years duration and moderate clinical signs with conventional therapy was given the FMT by enema, with 45 min retention time. Faecal consistency improved within 24 h and the dog had been clinically well at the time of the report (3 months after treatment). Next-generation 16S rRNA gene sequencing of the faecal microbiome revealed that by day 2 after FMT, the dogs' faecal sample clustered with the donor, not the own baseline sample, and species richness increased compared to pre-treatment values (Weese 2013). The other abstract reports the use of FMT in 8 dogs with refractory presumptive *Clostridium perfringens*-associated diarrhoea (Murphy *et al.* 2014). Again, it was administered as an enema (1–3 transplants per dog). All dogs had immediate resolution of their diarrhoea and 6/8 dogs were negative on follow-up PCR panels for *C. perfringens* alpha toxin. As mentioned before, *C. perfringens* can be part of the normal intestinal flora in dogs, hence it remains unclear if the detected *C. perfringens* was really the cause of the diarrhoea or rather a part of intestinal dysbiosis (Minamoto *et al.* 2014b). If that had been the case, it needs still to be critically assessed whether the FMT addressed Clostridiosis, even if the microbiome changes suggest an improvement of faecal dysbiosis. Both authors of the unpublished conference abstracts conclude that FMT should be considered as a treatment option in dogs failing other therapeutic options (Weese 2013; Murphy *et al.* 2014) and anecdotal evidence of its usefulness is increasing in the veterinary community. Prospective studies in large cohorts of dogs are needed to properly address the usefulness of FMT in defined GI diseases in dogs.

Conclusions

The microbiota on mucosal surfaces, especially the GI tract, in dogs is complex. The composition varies from site to site and there is some evidence that changes in the composition of the microbiota/microbiome are associated with certain diseases. However, analysis of the canine GI and faecal microbiota composition, its function, production of metabolites and immunological properties is far from complete, even though data on the microbiome are accumulating.

Accordingly, overall knowledge of the best characteristics of a canine microbial commensal or probiotic is patchy and most assumptions about their best properties are derived from human studies. There are some bacterial strains that show promise as potential probiotics, especially LAB. However, the effects of the most commonly used strain (*E. faecium*) are still not well understood, particularly in diseased dogs. It is challenging to compare outcomes of different studies, both in healthy and in diseased dogs, as there is huge variation in the probiotics used (single-strain, multi-strain, type of microorganism), their dosage, application form and frequency. Measured outcomes are also not consistent. Overall, it seems to the authors that *E. faecium* tends to be more suitable for acute and/or infectious forms of diarrhoea; as there is some evidence it produces a more pro-inflammatory rather than anti-inflammatory reaction (Schmitz *et al.* 2014, 2015b); whereas some lactobacilli and bifidobacteria show more pronounced immune-regulatory functions. Intriguingly, especially a combination of LABs (VSL#3) used in human medicine to prevent relapse of Ulcerative Colitis has some promise in treating chronic enteropathies in dogs and in creating a more anti-inflammatory local environment (Rossi *et al.* 2014). It is interesting to note that even in well-defined infectious diseases like parvovirosis or parasitic infestation, some probiotics show a beneficial effect. The mechanism behind this is not well understood and further research is warranted. It is clear from studies in people and experimental rodents, that the immunological outcome depends on both the bacterial strain or even subspecies – probably in a dose-dependent manner (Weese & Anderson 2002; Evrard *et al.* 2011; Garcia-Mazcorro *et al.* 2011) – and the respective host's immune response. In humans, probiotics have been classified into 'pro-inflammatory' and 'anti-inflammatory' by some authors, depending on their major properties (Shida *et al.* 2011). Because of this, careful assessment of potential probiotics in the target species and disease – possibly both *in vitro* and *in vivo* – is necessary to judge their full potential. Some authors even propose to test all probiotics *in vitro* or in tissue explants first, before performing *in vivo* trials (Tsilingiri *et al.* 2012). However, this might not always be possible in veterinary medicine.

There should also be a consideration that bacteria might not always be the most appropriate probiotics. We know virtually nothing regarding the fungal or even viral composition of the normal intestinal tract or other mucosal surfaces in dogs (Foster *et al.* 2013). There is some evidence that yeasts like *Saccharomyces boulardii* might be valid alternative probiotics and need more detailed investigations. Furthermore, an emerging field is the research into bacterial metabolites (e.g. indole, acetate) that may potentially serve as postbiotics.

Faecal transplantation is an interesting option to treat both acute and chronic diarrhoea in dogs, however, much more needs to be understood regarding its best performance, safety and usefulness, before it can be recommended as a routine treatment.

In summary, more work needs to be done to understand the complex interplay between potential probiotics and their host environment. Very careful investigations into mechanisms of action and detailed measured outcomes will help to understand which probiotic is useful in which condition, and which changes of the intestinal microbiome are necessary to achieve clinical remission of both acute and chronic conditions.

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