Dysbiosis, antibiosis and probiosis – diarrhoea and the microbiome

Iwan A. Burgener, Prof. Dr.med.vet., PhD,
Dr. habil, Dipl. ACVIM & ECVIM-CA

Head of Small Animal Internal Medicine and Head of Small Animal Clinic
VetMedUni Vienna, Veterinaerplatz 1, 1210 Vienna, Austria
iwan.burgener@vetmeduni.ac.at

Microbiota / microbiome and the interaction with the immune system

Humans and animals live together in a cross-species life form (biocenosis) with 100 trillion bacteria. The totality of the organisms (besides bacteria also archaea, viruses, fungi and protozoa) forms our microbiota. It colonizes all our interfaces to the environment and, together with genetic information and metabolism, is called microbiome. We are therefore never alone as an individual and expand as a "super-organism" our cell count by a factor of 10, the genetic information even by a factor of 150. Although the microbiome is not a heavyweight, it still makes up almost 2 % of our body weight - similar to the brain (1).

In recent years, the findings on the nature and impact of this biocenosis explode. In particular, the sustainable and profound integration of the microbiome with the immune system is surprising. This opens the door to completely new pathophysiological contexts - including the influence on diseases via the environment - for example through nutrition and antibiotics - becomes understandable. Prerequisite for these new insights are always cheaper and faster molecular diagnostic methods such as pyrosequencing. This allows the entire DNA of a sample to be sequenced. This creates enormous amounts of data that only become interpretable and understandable with the aid of bioinformatic methods. In addition to the host genome, the metagenome appears in the samples analysed this way. It contains the genetic information of a large number of new bacterial species, which remain hidden to more than 80% when creating classical bacterial cultures. In addition to the genetic information in the microbiome, also metatranscriptome (RNA), metaproteome (proteins) and the metabolome (metabolites) can be analysed.

The gastrointestinal tract of a foetus is sterile in utero. Under the onset of environmental influences such as habitat, hygiene, vaccinations, nutrition and antibiotic exposure, the adult microbiota, which is largely stable in composition, develops during the first year of life. Four strains dominate the bacterial microbiota: Gram-positive Firmicutes and Actinobacteria as well as Gram-negative Proteobacteria and Bacteroidetes. In recent years, the crucial role of dialogue with the body's immune system is becoming increasingly clear.

Immune functions develop essentially only under the influence of the microbiota. Mice growing up sterile are immunosuppressed. Lymphoid follicles under the intestinal epithelium only form in the presence of the microbiota in the lumen. However, the microbiota not only affects the intestinal portion of the immune system - the immunological signals from the intestinal lumen reach the entire organism and are detectable even in the central nervous system (gut brain axis).

Eubiosis, dysbiosis, nutrition and antibiotics in human medicine

Much is still unclear regarding the influence of individual bacterial species on disease and health. However, it is known that a large bacterial diversity is the expression of a healthy microbiota ("eubiosis"). A dysbiosis with reduced diversity and predominance of individual bacterial species, however, is found in a variety of diseases. In humans, these include obesity, inflammatory bowel disease (IBD) and Clostridium difficile-associated diarrhoea, as well as extra-intestinal diseases such as allergies and asthma as well as autoimmune diseases, but also neuropsychiatric disorders. Currently, one question remains unanswered: is dysbiosis in a disease an expression of causality, or is it merely an association or correlation? At least in animal experiments, there are clear indications of a causal relationship. If sterile mice (germ-free, GF) are colonized by microbiota of obese mice or humans, the recipient mice - regardless of diet - also become obese. However, if lean mice and their microbiota are in the same cage at the same time as the GF mice, the diet determines whether a GF mouse becomes obese or stays slim (2). In short, obesity can be transplanted via the microbiota in a controlled animal model. It is intuitively understandable that the type of diet also directly influences the composition of the microbiota and thus the eubiosis. For example, Prevotella predominantly distinguishes a "vegetarian enterotype" from a "carnivor enterotype" with dominance of Bacteroides. Furthermore, the microbiota is complementary in that essential micronutrients (e.g., folic acid or riboflavin), if not present in the diet, are increasingly produced by the microbiota.

The discovery of the profound interaction between organism and microbiome and an association between dysbiosis and a variety of diseases raises hope for a therapeutic influence. The "resetting" of the microbiota into eubiosis could also favourably influence or even cure the disease. In fact, with the transplantation of eubiotic microbiota (faecal transplantation = FT), there is a kind of proof of concept that supports this assumption. So far, FT has been successfully used in recurrent infections with Clostridium difficle (RCDI). The treatment is effective in over 90 % of cases and thus decidedly superior to the previous treatment option with antibiotics (3). Because antibiotic therapy is the best-known risk factor of RCDI, faecal storage for later auto transplantation (auto banking) prior to antibiotic therapy is also discussed. Any use of antibiotics inevitably leads to the reduction of the metagenome with microbial dysbiosis and the selection of antibiotic-resistant bacteria (1). So far, an altered microbiota has been documented over a period of up to four years (4). The principle of any therapeutic intervention, namely "primum non nocere", so receives a new weight for each antibiotic use.

Finally, the combination of bacteriotherapy and nutrition as well as pre-, pro- or synbiotics has long been available in human medicine. However, different species, combinations and dosages have led to few indications being clearly proven in humans. Therapeutically, this applies only to the acute infectious diarrhoea. Prophylactically, its efficacy is demonstrated in neonatal necrotising enterocolitis, acute antibiotic-associated diarrhoea, pouchitis and lactose intolerance (5).

Antibiotics and dysbiosis in small animals?

More than 20 years ago, it was thought that overgrowth of the physiological intestinal bacteria in the small intestine (Small Intestinal Bacterial Overgrowth, SIBO) could lead to diarrhoea. However, compared to humans, the small intestine of the dog is relatively heavily populated and there are dogs that exceed the limit without clinical symptoms. Recent studies indicate that simple overgrowth is insufficient to describe pathophysiological relationships - it is rather a shift in the bacterial ecosystem in the small intestine. Antibiotic-responsive diarrhoea (ARD) and tylosin-responsive diarrhoea (TRD) are newer terms that are likely to describe similar diseases.

Antibiotics can sometimes provide relief in dogs and cats with gastrointestinal disease. This suggests that bacteria have an etiological significance in these patients. However, it has to be differentiated that there are patients who have disease due to enteropathogenic germs (e.g., enteroinvasive E. coli, probably Campylobacter jejuni). However, there are also germs that can be found in the gastrointestinal tract of healthy dogs and cats, and lead to gastrointestinal diseases only under certain conditions (e.g., Clostridium perfringens and Clostridium difficile). There are also gastrointestinal diseases, which respond to antibiotics despite undetectable (potentially) pathogenic germs. These patients are considered to have dysbiosis (dysbacteria, shift in the bacterial ecosystem). The reasons for such dysbiosis are manifold. In most cases, dysbiosis is secondarily associated with other diseases (e.g., pancreatic exocrine insufficiency, IBD, dysmotility, stasis, endocrine disorders, decreased acid production in the stomach, partial obstructions (neoplasia, foreign body, intussusception)), but it may also be primary.

Diagnosis of dysbiosis

Exact criteria for the diagnosis of dysbiosis in the intestinal tract do not exist. Neither the bacteriological examination of a faecal sample nor that of small intestinal juice permits a diagnosis. An increase in the serum folate concentration (bacteria can produce folic acid), a decrease in serum cobalamin (bacteria bind and consume cobalamin), as well as a successful treatment of suspected dysbiosis suggest a possible dysbiosis. Recent studies in dogs (6-9) have demonstrated with pyrosequencing that in acute and chronic diarrhoea, IBD, food-responsive diarrhoea (FRD) and intestinal lymphoma, similar changes in gut microbiota are found as in human dysbiosis (less diversity, shift of groups). The most massive shifts in faecal samples were seen in dogs with acute haemorrhagic diarrhoea together with an increase in Clostridium perfringens (6). Furthermore, mucosal samples from patients with IBD show significant differences to healthy dogs before therapy (7) and between IBD and FRD before and after therapy (9). However, the results are sometimes confusing and contradictory (10).

Fluorescence in situ hybridization (FISH) has shown an increase of Enterobacteriaceae and E. coli on or in the intestinal mucosa of dogs with IBD or granulomatous colitis („Boxer colitis“; 11). An attempt to establish a human dysbiosis index for canine IBD failed in spite of similar shifts (12) and led to the establishment of a faecal canine dysbiosis index (12,13). However, all of the methods described here (6-13) are currently not accessible for routine diagnostics.

To make matters worse, with sequencing we can only say whether the bacteria are present or not. It would be much more important to know in which quantities they are present, whether they are still alive, which products they produce for their environment and which metabolites may be measurable in serum as biomarkers. First (hesitant) attempts in this direction already exist (14-16), but have so far not left noticeable traces in everyday clinical practice.

Therapy of dysbiosis

The aim of treating (suspected) dysbiosis is to restore the physiological microbiota. For this purpose, pre-, pro-, syn- or antibiotics can be used.

Prebiotics

- support the physiological bacterial ecosystem
- indigestible food components are fermented
- short-chain fatty acids are produced as an energy carrier for colonic mucosa
- best-known example: fructooligosaccharides (FOS)

Probiotics

- live bacterial strains, part of the physiological gut microbiota
- requirements
  - must be safe and stable
  - positive effect on the animal
- number of scientific papers on effectiveness very limited
- uncontrolled studies: positive effect on stress diarrhoea

Synbiotics

- combinations of pre- and probiotics
- used partly by dog food manufacturers
- more promising: joint use (prebiotics in the food, probiotics orally)

Antibiotics?

- Tylosin (25 mg/kg q12-24h PO)
  - not used in human medicine
  - practically no side effects
  - disadvantage: bitter taste (possibly capsules), recurrence often
- Metronidazole (10-20 mg/kg q8-12h PO)
  - more side effects

With a diagnosis of low cobalamin, it may also be helpful to supplement cobalamin. Typically, cobalamin is injected once a week for the first 6 weeks, then depending on the control results. The recommended dosages are 250ug (cats and dogs <5kg), 400ug (dogs 5-15kg), 800ug (15-30kg), 1200ug (30-45kg) and 1500ug for dogs> 45kg. Oral therapy in dogs with chronic enteropathies is also established and effective.

Probiotics and dysbiosis – what do we really know by now?

One of the first clinically relevant studies is over 10 years old. Dogs with FRD were treated with a probiotic cocktail of 3 Lactobacillus strains in a prospective, placebo-controlled, double-blind study (17). After treatment, duodenal mRNA of IL-10 and that of INF-γ in the colon increased. The number of lactobacilli in the faeces increased during therapy, that of Enterobacteriaceae declined - but in both groups.

Although probiotics have been widely used in the last decade, clinically relevant studies in the dog have only been published since 2014 (18-21). In a very small open study, the efficacy of the probiotic VSL # 3 (Sivoy, Streptococcus thermophilus, Bifidobacteria spp., lactobacillus spp.) in IBD was tested and compared to prednisolone and metronidazole (60 days therapy; 18). Thirty days after the end, both groups were clinically and histologically significantly better, with the VSL # 3 group yielding a significantly higher number of regulatory T cells (FoxP3 +, TGF-β +) and faecalibacteria. A prospective, placebo-controlled, double-blinded study with Enterococcus faecium (Synbiotic D-C, Protexin) in FRD showed no measurable difference between placebo and probiotics (19), potentially because many dogs unfortunately did not complete the study. In a randomized multicentre trial in the USA, 34 dogs with IBD received either standard therapy (ST = food and prednisolone) or ST plus probiotics (Visbiome or Vivomixx, similar to Sivoy). The groups were compared with mucosal FISH initially and in faecal samples after 8 weeks of therapy (20). Both treatments increased the total number of bacteria and their species. There were also very few other differences, with the probiotics group showing a higher expression of certain proteins that could have a positive effect on mucosal homeostasis (tight junction protein, E-cadherin, occludin, zonulin). In the latest study, another probiotic was used in a prospective, placebo-controlled, double-blinded study to treat 20 dogs with chronic enteropathy with ST +/- Saccharomyces boulardii (non-pathogenic yeasts; 21). After 2 months of therapy, the S. boulardii group showed significantly better clinical disease status (CCECAI), stool frequency, stool consistency and body condition score compared to ST.

In summary, the evidence suggests that certain probiotics (mixed products are likely to be better) may be helpful in certain clinical problems (chronic enteropathy, FRD, IBD) when used long enough in addition to the ST. Some effects only occurred after 30 to 60 days. In addition, there are basic studies, which also prove that most bacterial strains used today can at least temporarily settle in the canine intestine. Probiotics are not always effective, but they probably never hurt - something you cannot say about antibiotics in connection with gastrointestinal problems.

Literature

20. Kahlert C, Müller P: Mikrobiom – die Entdeckung eines Organs. Schweiz Med Forum 14(16–17):342–344, 2014.
21. Ridaura VK, Faith JJ, Rey FE, et al: Gut Microbiota from Twins Discordant for Obesity Modulate Metabolism in Mice. Science 341(6150):1241214, 2013.
1. Van Nood E, Vrieze A, Nieuwdorp M, et al: Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med 368(5):407–15, 2013.
2. Jakobsson HE, Jernberg C, Andersson AF, et al: Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PloS One 5(3):e9836, 2010.
3. Sanders ME, Guarner F, Guerrant R, et al: An update on the use and investigation of probiotics in health and disease. Gut 62(5):787–96, 2013.
4. Suchodolski JS, Markel ME, Garcia-Mazcorro JF, et al: The fecal microbiome in dogs with acute diarrhea and idiopathic inflammatory bowel disease. PLoS One 7(12):e51907, 2012.
5. Suchodolski JS, Dowd SE, Wilke V, et al: 16S rRNA gene pyrosequencing reveals bacterial dysbiosis in the duodenum of dogs with idiopathic inflammatory bowel disease. PLoS One 7(6):e39333. doi:10.1371/journal.pone.0039333, 2012.
6. Omori M, Maeda S, Igarashi H, et al: Fecal microbiome in dogs with inflammatory bowel disease and intestinal lymphoma. J Vet Med Sci 79(11):1840-1847, 2017.
7. Kalenyak K, Isaiah A, Heilmann RM, et al: Comparison of the intestinal mucosal microbiota in dogs diagnosed with idiopathic inflammatory bowel disease and dogs with food-responsive diarrhea before and after treatment. FEMS Microbiol Ecol, 94, fix173, 2018.
8. Honneffer JB, Minamoto Y, Suchodolski JS: Microbiota alterations in acute and chronic gastrointestinal inflammation of cats and dogs. World J Gastroenterol Nov 28;20(44):16489-97. doi: 10.3748/wjg.v20.i44.16489, 2014.
9. Cassmann E, White R, Atherly T, et al: Alterations of the Ileal and Colonic Mucosal Microbiota in Canine Chronic Enteropathies. PLoS One 11(2):e0147321, 2016.
10. Vazquez-Baeza Y, Hyde ER, Suchodolski JS, Knight R: Dog and human inflammatory bowel disease rely on overlapping yet distinct dysbiosis networks. Nat Microbiol 1:16177, 2016.
11. AlShawaqfeh MK, Wajid B, Minamoto Y, et al: A dysbiosis index to assess microbial changes in fecal samples of dogs with chronic inflammatory enteropathy. FEMS Microbiol Ecol, 93, fix136, 2017.
12. Minamoto Y, Otoni CC, Steelman SM, et al: Alteration of the fecal microbiota and serum metabolite profiles in dogs with idiopathic inflammatory bowel disease. Gut Microbes 6:1,33-47, 2015.
13. Guard BC, Barr JW, Reddivari L, et al: Characterization of Microbial Dysbiosis and Metabolomic Changes in Dogs with Acute Diarrhea. PLoS One 10(5):e0127259, 2015.
14. Guard BC, Suchodolski JS: Canine intestinal microbiology and metagenomics: From phylogeny to function. J Anim Sci 94:2247-2261, 2016.
15. Sauter SN, Benyacoub J, Allenspach K, et al: Effects of probiotic bacteria in dogs with food responsive diarrhoea treated with an elimination diet. J Anim Physiol Anim Nutrition 90,269-277, 2006.
16. Rossi G, Pengo G, Caldin M, et al: Comparison of Microbiological, Histological, and Immunomodulatory Parameters in Response to Treatment with Either Combination Therapy with Prednisone and Metronidazole or Probiotic VSL#3 Strains in Dogs with Idiopathic Inflammatory Bowel Disease. PLoS One 9(4):e94699, 2014.
17. Schmitz S, Glanemann B, Garden OA, et al: A Prospective, Randomized, Blinded, Placebo-Controlled Pilot Study on the Effect of Enterococcus faecium on Clinical Activity and Intestinal Gene Expression in Canine Food-Responsive Chronic Enteropathy. J Vet Intern Med 29:533-543, 2015.
18. White R, Atherly T, Guard B, et al: Randomized, controlled trial evaluating the effect of multi-strain probiotic on the mucosal microbiota in canine idiopathic inflammatory bowel disease. Gut Microbes 8(5):451-466, 2017.
19. D'Angelo S, Fracassi F, Bresciani F, et al: Effect of Saccharomyces boulardii in dogs with chronic enteropathies: double-blinded, placebocontrolled study. Vet Rec doi:10.1136/vr.104241, 2018.