



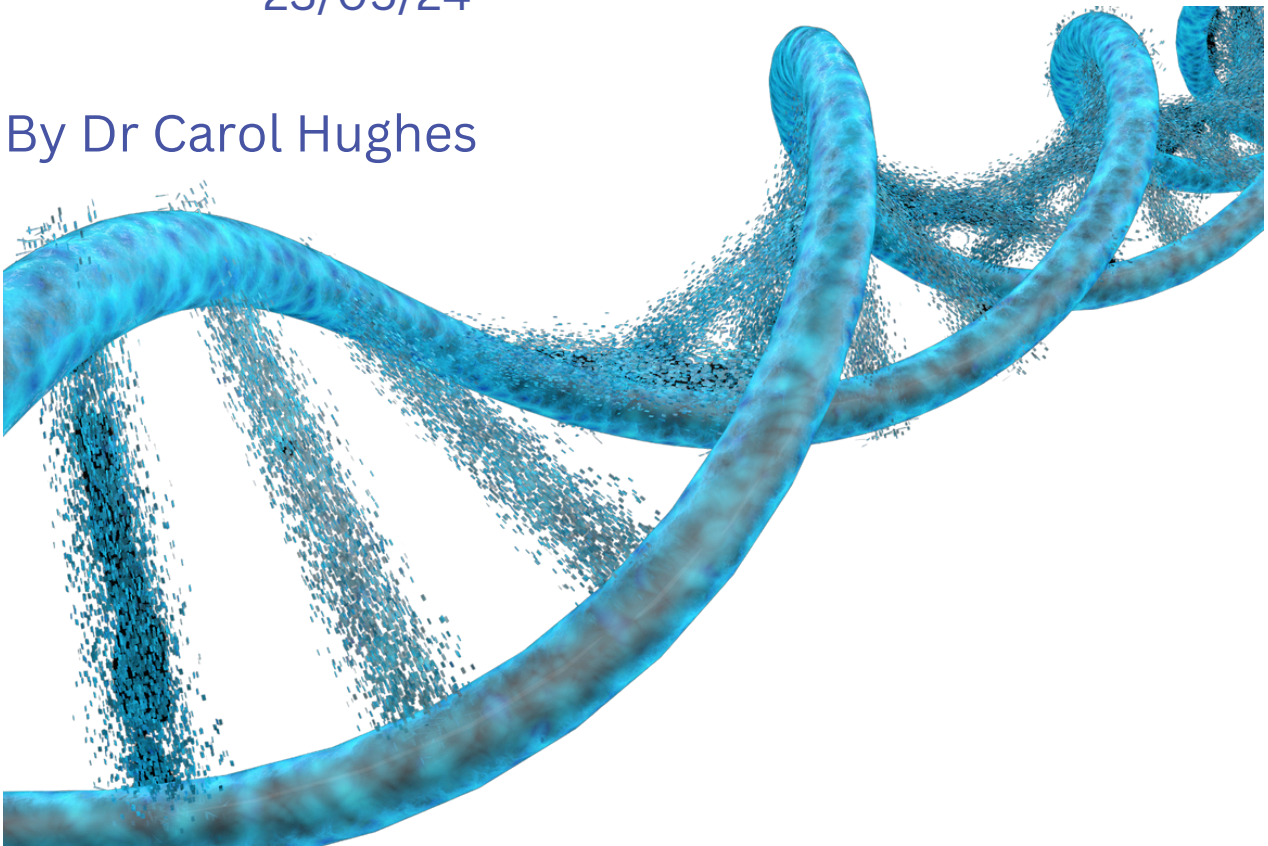
BIOME4PETS
Microbiome Testing Service

Petbiome Microbiome Profile

Prepared Personally For

Sample
23/05/24

By Dr Carol Hughes



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That help to digest nutrients from the diet, having enough of each indicates the diet is working well for your dog.

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The Microbiome

The microbiota comprises a community of bacteria that live in the gut of all mammals that are species specific, this report relates to the dog.

The Good Gut Bacteria

Make vitamins and allow minerals to be absorbed.

Mend the gut wall and prevent ulcers and inflammation.

Defend against the invasion of bad bacteria Increase energy and promote a good immune response.



The Bad Gut Bacteria

Cause disease -colitis, IBD, SIBO, gastritis, inflammation food sensitivities and allergies. Create imbalances, triggered by changes in diet, stress, commonly used medication, including the use of antibiotics and pain medication.

Rebalancing the Gut

Is much easier if you know what and where these imbalances are. Scientific research has linked every common gastrointestinal health problem to the gut bacteria and this report will identify each one and give advice on how to restore balance.

3 Common States of the Biome

1.A Balanced Microbiome

2.A biome in the state of dysbiosis or imbalance, too many bad bacteria, good bacteria present but in too few numbers.

3. Complete absence of good gut bacteria.

More about the Microbiome

The Report

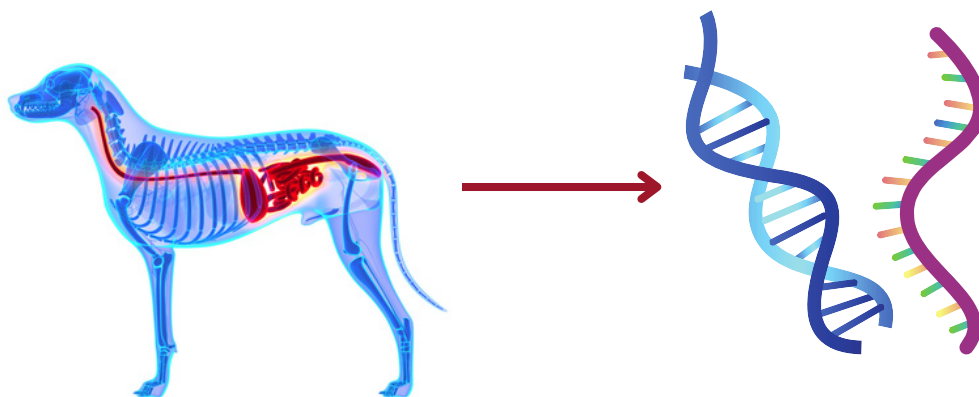
This report is an analysis of the 16S rRNA gene which is present in all bacteria and is the most accurate method of identifying bacterial species. It provides a real time snapshot of the hindgut microbial community of your dog.

Whilst the test is a powerful management and analytical tool, it is not intended to be used to diagnose any illness, please consult your vet if your dog is in discomfort. To generate this report The Illumina MiSeq is used, which is the most accurate and up to date technology, chosen by genomic researchers around the world.

In dogs, the knowledge and science linking microbiome to health and disease, are in their infancy. In humans this area of science is much more advanced because of the larger database of samples. It is our aim to gather as many samples of different groups (populations) of dogs as we can, this will help to increase our knowledge and put it in line with human research, offering the best and most accurate service.

The PetBiome Data Library

We have the largest library of canine microbiome data in the world, used to identify and accurately describe the healthy biome. The data base consists of faecal samples from dogs of all ages and breeds. We then profile the microbiome using artificial intelligence and population data. Since 2007 we have collected thousands of samples from dogs with obesity, allergies, arthritis, gastric discomfort, inflammatory bowel disease, diarrhoea and more. We have also profiled the microbial communities of dogs on different diets and work with many vet practices profiling disease and dysbiosis. The use of long term medication can have a profound effect on the microbiome most commonly antibiotics, anthelmintics, steroids and non steroidal anti-inflammatories, use of these medications has the effect of reducing diversity, we have worked for many years on how to restore the extensive and diverse microbial community through dietary management and the use of plant compounds and secondary metabolites.



The Petbiome analysis sequences the rRNA of the gut bacteria to give information in real time

The Duodenum

The absorption of vitamins, minerals, and other nutrients begins in the duodenum, aided by the firmicutes which are highest in this part of the gut.

Cyanobacteria, Lactobacillus, Faecalibacterium, and Ruminococcus are also found in the duodenum

Sections of your report relating to the microbes in this are is-

Fibre digestion, bloating Immune function, metabolism and inflammation

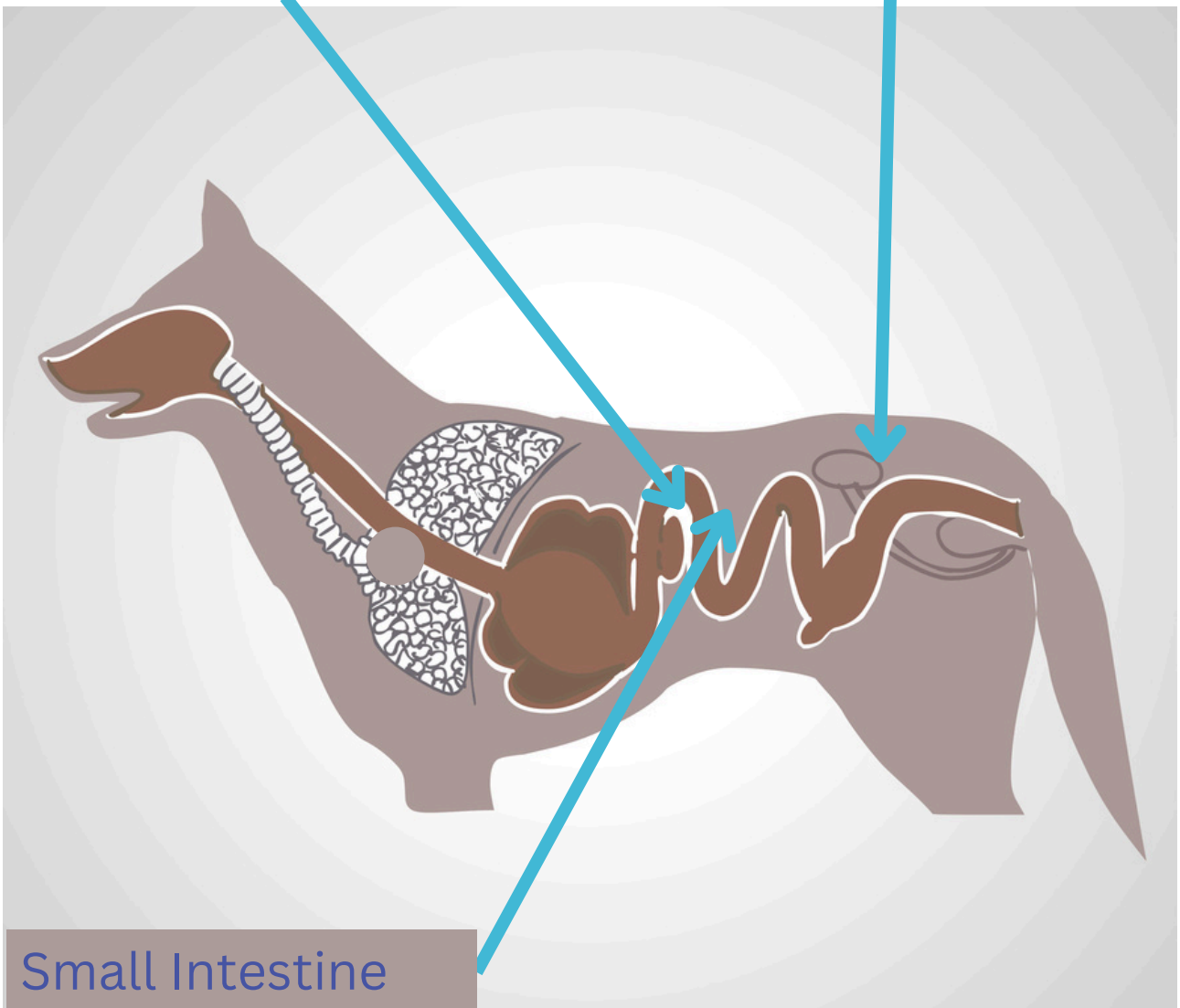
The Large Intestine

Anaerobes predominate in the large intestine, identified as Firmicutes, Bacteroides, and Fusobacteria

Residents within this part of the gut are prolific producers of butyrate, known to have a protective role in the gut.

Sections of your report relating to the large intestine include-

Bloating and hydrogen production



Small Intestine

The small intestine is home to Clostridia, Lactobacillales, and Proteobacteria being most abundant. Protein, fat, carbohydrate, trace elements, vitamins, and water are mainly absorbed in this part of the G.I. tract.

Excessive bacteria in the small intestine causes a disorder called SIBO with symptoms including (diarrhea, gas and vomiting).

Sections of your report relating to the small intestine include



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Part 2. Your Dog

This section contains information relating to the core state of the microbiome, how stable, diverse and resilient it is.

Veterinary Summary

This part of the report contains the most important information relating to the health of your dog. Many gastric imbalances linked to discomfort/disease are caused by 'bad' gut bacteria that make toxins, translocate across the gut wall, cause inflammation and are antibiotic resistant.

We have identified multiple combinations of dysbiotic bacteria profiles, which when rectified causes a knock on improvement to the rest of the microbiome and a visual improvement in the health of the dog.

Before making any other of the other recommended changes to the diet, please follow the advice on this page first, as once the primary dysbiosis/imbalance is rectified there may be no need to take any of the other recommended actions. Please contact us carol@biome4pets.com if you are unsure and we'll be happy to talk it through in more detail.

Below is a summary of any dysbiosis that exists in your dog together with advice on how to restore balance.

Your pet has an overgrowth of Clostridium Perfringens present at 5% of the total biome. The average in healthy dogs is 0.003 to 0.01%. C. Perfringens produces two toxins known to cause diarrhea. The toxins produced can ulcerate the intestinal lining.

Levels of fusobacterium mortiferum are higher than average, usually present in the microbiome of healthy dogs at 0.1% but in this analysis is present at 20% of the total biome. Fusobacteria are a normal core part of the microbiome of all dogs but imbalances do occur which cause gastric discomfort and food sensitivities.

Fusobacteria use amino acids as fermentable energy sources to produce beneficial secondary metabolites such as acetic, butyric and propionic acids. In contrast to other fusobacteria species, F. mortiferum ferments an extraordinarily wide variety of carbohydrates and has been cited in the development of metabolic dysregulation of humans ie type 2 diabetes. F. mortiferum produces Phospho- α -glucosidase an enzyme which helps increase the uptake of carbohydrates and sugars.

To reduce F.mortiferum a good soil pro/prebiotic such as Four Leaf Rover Protect is recommended as it contains bacteria that manufactures ascorbate which will make the microbiome less favourable for the production of F. mortiferum and the production of the enzymes.

Gohari, I. M., Parreira, V. R., Nowell, V. J., Nicholson, V. M., Oliphant, K., & Prescott, J. F. (2015). A novel pore-forming toxin in type A Clostridium perfringens is associated with both fatal canine hemorrhagic gastroenteritis and fatal foal necrotizing enterocolitis. PLoS one, 10(4), e0122684.

Diversity Score

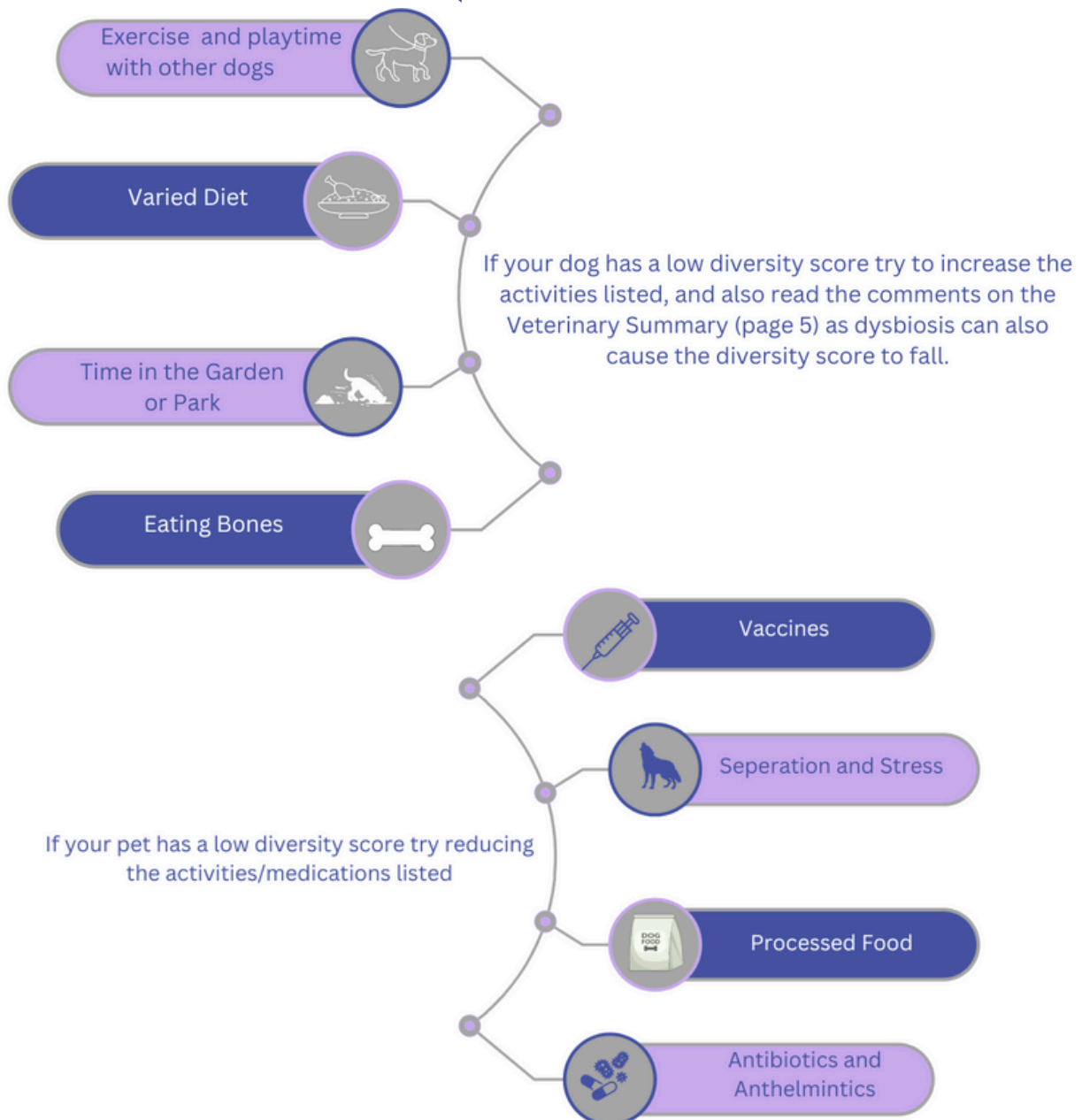
Low < 1.9

Medium 2.1-2.5

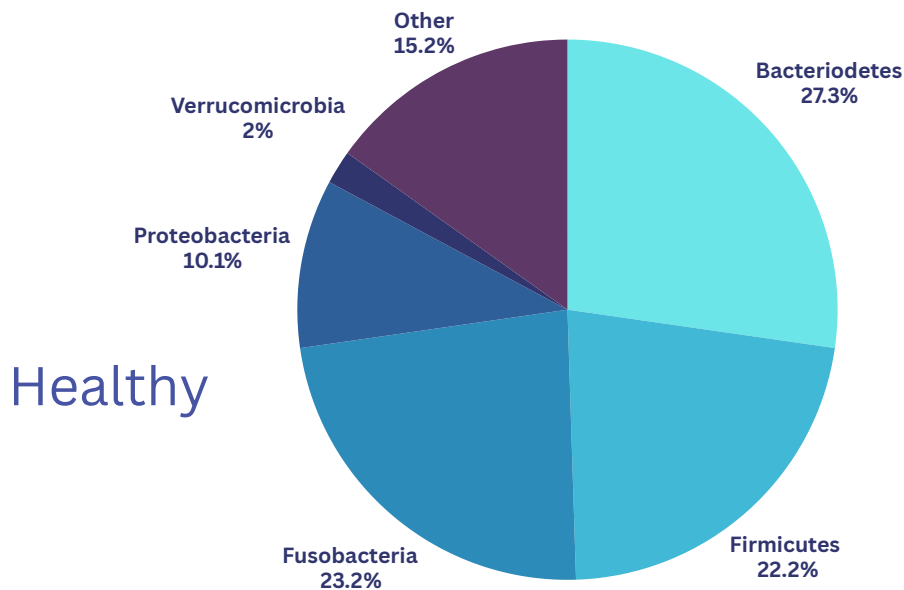
High > 2.5

Your Pet 2.5

Diversity scores are calculated using the Shannon Index Equation



Biome Analysis

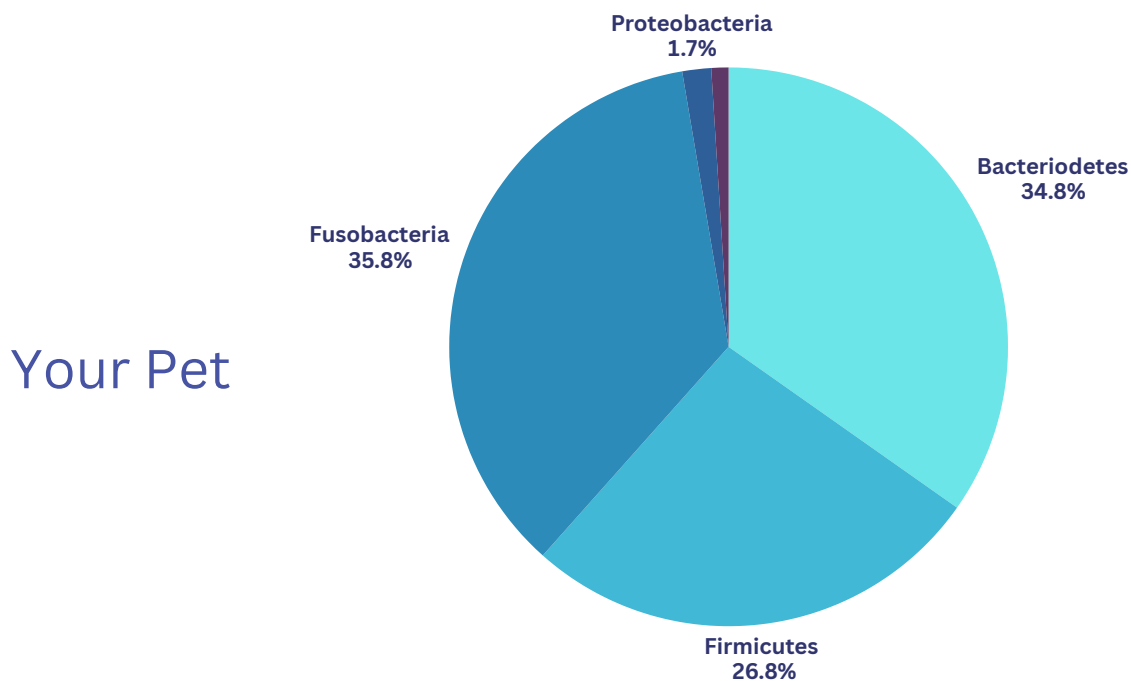


The Core Members of the Biome

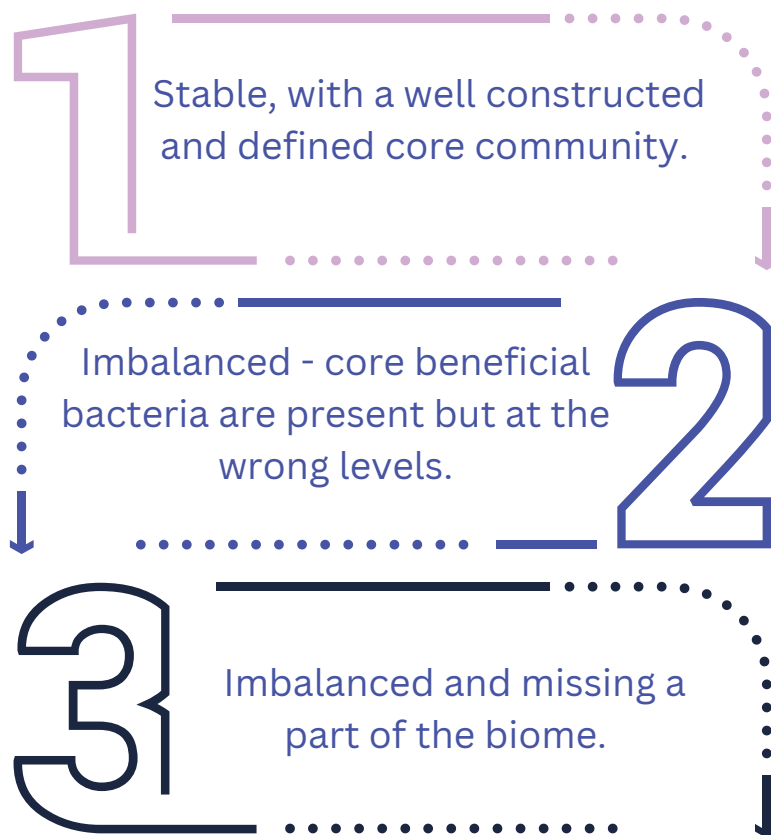
The core microbiome is a set of consistent microbial features across populations. Understanding this core can help pinpoint factors contributing to dysbiosis.

These are important for the health of the dog because they interact with the dog's immune system and metabolic/energy cycles.

These bacteria are also involved in the digestion of nutrients and the health of the gastrointestinal tract. .



The Three Biome States



This information has been calculated from the identification of thousands of species of bacteria from within your dogs gut, which is then compared to the phylotypes of healthy/unhealthy dogs within our data base.

Your Pet

An Imbalanced and Missing part of the Biome

Imbalanced

The core members of the microbiome are present but at the wrong levels described as dysbiosis. There is an increased opportunity for inflammation and infection to develop and also allergies, food sensitivities, IIBS etc. Dogs with this type of microbiome are sensitive to changes in management and diet.

Missing There are specialized bacteria responsible for protecting the host against invading pathogens, environmental pollutants, and environmental bacteria. Others are linked to energy production and the endocrine system. These bacteria are either extremely low or missing altogether. Causes can be the use of long- term medication such as NSAID's, omeprazole or antibiotics, processed food, eutrophication (high mineral supplements) and some anthelmintics. It is possible to repopulate the gut with good gut bacteria though for some dogs recovery can take some time.

Section 2. 5 Important Microbes Associated with Diet

Bacteroidales

Bacteroidales have multiple roles and are often antibiotic-resistant. Some are digesters of carbohydrates, some are digesters of mucin and heparin (found in meat products, especially raw) and around 19% are pathogenic.

Bacteroidales are complex and have a generally beneficial relationship with their canine host when retained in the gut, however, if they cross the gut barrier, they can cause infections and abscesses in other parts of the body.

Bacteroidales possess complex systems to sense and adapt to nutrient availability, multiple pump systems to expel toxic substances and can alter the host immune system to control other competing pathogens.

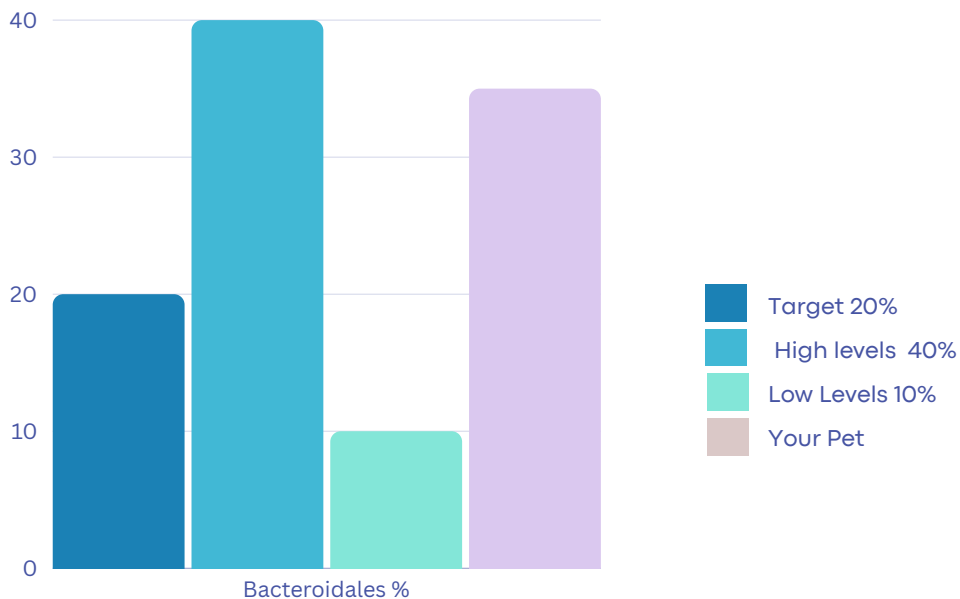
Clinically, Bacteroidales species have exhibited increasing resistance to many antibiotics, including cefoxitin, clindamycin, metronidazole, carbapenems, and fluoroquinolones (e.g., gatifloxacin, levofloxacin, and moxifloxacin).

A higher percentage of resistant bacteroidales will result in an overgrowth during antibiotic use with an increased possibility of a post antibiotic infection.

Dietary Advice

The graph below will identify whether there are high levels of bacteroidales and will also identify whether the dog is able to digest carbohydrates or animal products.

If levels are low, check your dog's diet for foods that contain threonine, serine, proline, and cysteine (available in meat, whole grains and cheese) these will help to produce mucin which makes the biome more favourable for bacteroidales.



Ability to digest carbohydrates

High

Ability to digest meat/offal

Low

Pathogenic/antibiotic-resistant Bacteroides

Medium

Fusobacteria

Fusobacteria are an important member of the healthy canine gut, being the third most common bacteria in meat eating healthy dogs.

Not much is known about the role of the phyla fusobacteria in dogs but from our population data base there are three sub division species that predominate.

The first is *Fusobacterium perfoetens* reported as being much higher in dogs that are overweight, this bacteria converts glucose to lactic acid.

Glucose is an ingredient that can be listed as sugar, caramel, syrup or sucrose and found in corn/maize, wheat, sugar cane and sugar beet.

The second is *Fusobacterium mortiferum*, linked to infections and discomfort.

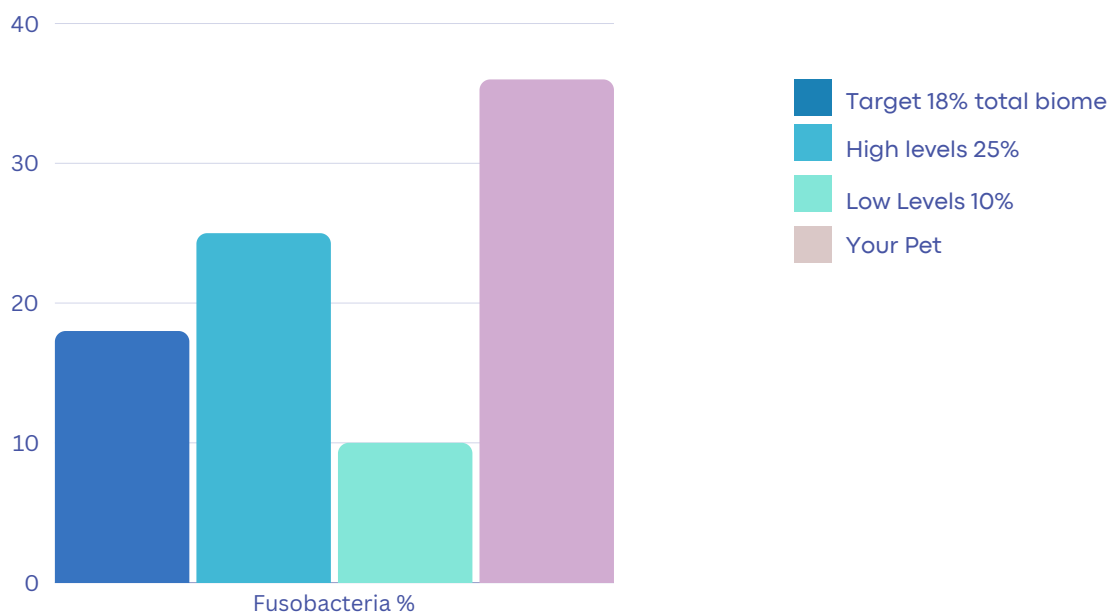
The third common component of the phylum fusobacteria is *Fusobacterium*, this genus increases in dogs with high access to the outside ie. garden, fields and parks.

Please read the veterinary summary (page 5) which will highlight imbalances between the species and highlight species that may be detrimental to health.

Fusobacteria gets its energy from fermenting select carbohydrates and amino acids. In low numbers it has been linked to colitis and inflammatory bowel disease. Increasing the amino acid content could help to increase levels.

Look on the food labels for the ones that aren't manufactured by the dog's own body but must be added into their daily feed. The ten essential amino acids that the diet should provide to help increase the fusobacterium are- arginine, histadine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine.

Chun, J. L., Ji, S. Y., Lee, S. D., Lee, Y. K., Kim, B., & Kim, K. H. (2020). Difference of gut microbiota composition based on the body condition scores in dogs. *Journal of animal science and technology*, 62(2), 239.



Prevotella

Prevotella are part of the normal healthy biome and contribute to the health of the dog by helping to digest carbohydrates and protein, they also produce an important supply of energy. An overgrowth (high levels), can then be linked to infections of the gastrointestinal tract, which will be reported on the veterinary summary (page 5).

In dogs 93% of the prevotella are identified as copri, this species increases the risk of arthritis and other inflammatory conditions. Prevotella levels increase in diets high in complex carbohydrates but low in protein. Slightly increasing the complex carbohydrate content of the dog's diet should help raise Prevotella and improve overall glucose metabolism.

Prevotella is considered to be a biomarker or measure of how much fibre is in the diet, but when levels that are too high, there is a link to inflammation.

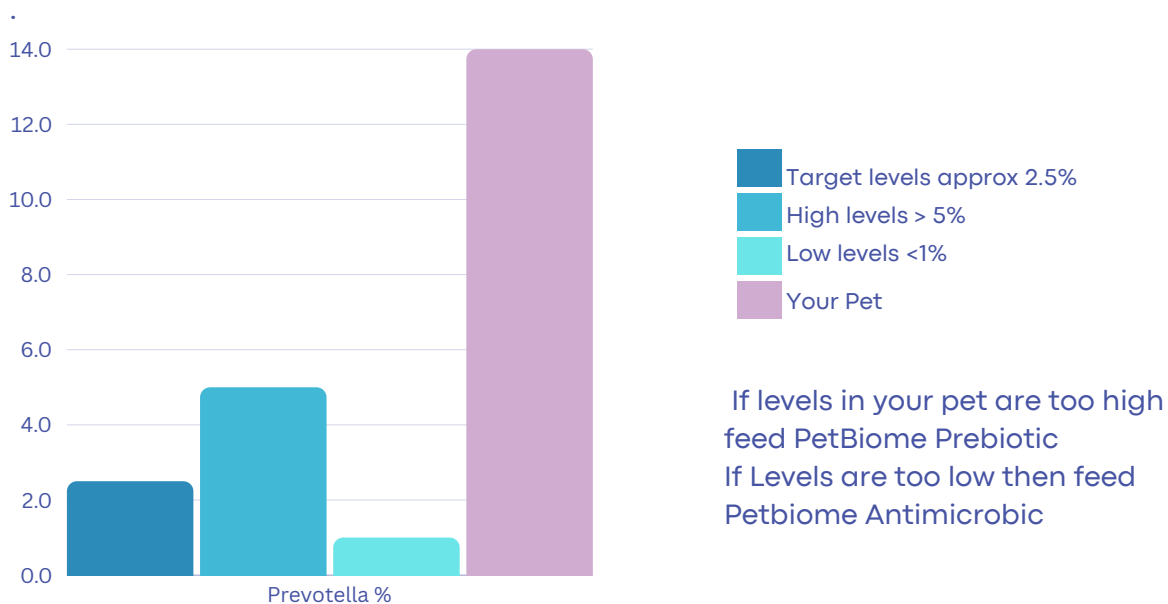
The prevotella/bacteroides ratio is important as when both are at target levels the carbohydrate/fibre intake of the dog is considered to be balanced

Prevotella helps to breakdown protein and fibre in a diet. If present in high numbers they can become opportunistic pathogens.

Dietary Advice

If levels are too low then increase the fibre part of the diet, avoid processed fibre listed as cellulose powder on the ingredient list. Cellulose, hemicelluloses, and lignins from the skins of fruit or vegetables are examples of insoluble fiber. They are not digested and pass through the gut essentially unchanged.

Complex carbohydrates also help to increase Prevotella, found in many formulated dog foods, include unprocessed grains, oat bran, hulls of brown rice, and beet pulp or prebiotics containing arabinoxylan. Avoid 'grain-free' feeds as these contain carbohydrates such as tapioca, sweet potatoes and potatoes but don't have the type of complex carbohydrates required. (Neyrinck et al 2011)



Clostridia

Clostridia are a very important component of the biome, the recommended average is 15%. Class clostridia, from the phyla firmicutes, contain many different species of bacteria, of which a few are pathogens (botulin), overgrowths are linked to diseases such as acute hemorrhagic gastroenteritis .

The majority, however, are very host friendly. Clostridia form part of the biome 'police force' defending the gut wall barrier against invading bacteria, they also signal for an immune response and interact with other bacteria within the biome, releasing friendly feel-good chemicals to help bond the microbial community.

Clostridia help initiate an immune response in many parts of the body but one of the most important is the link to respiratory health through a shared mucous membrane along the gut/lung axis.

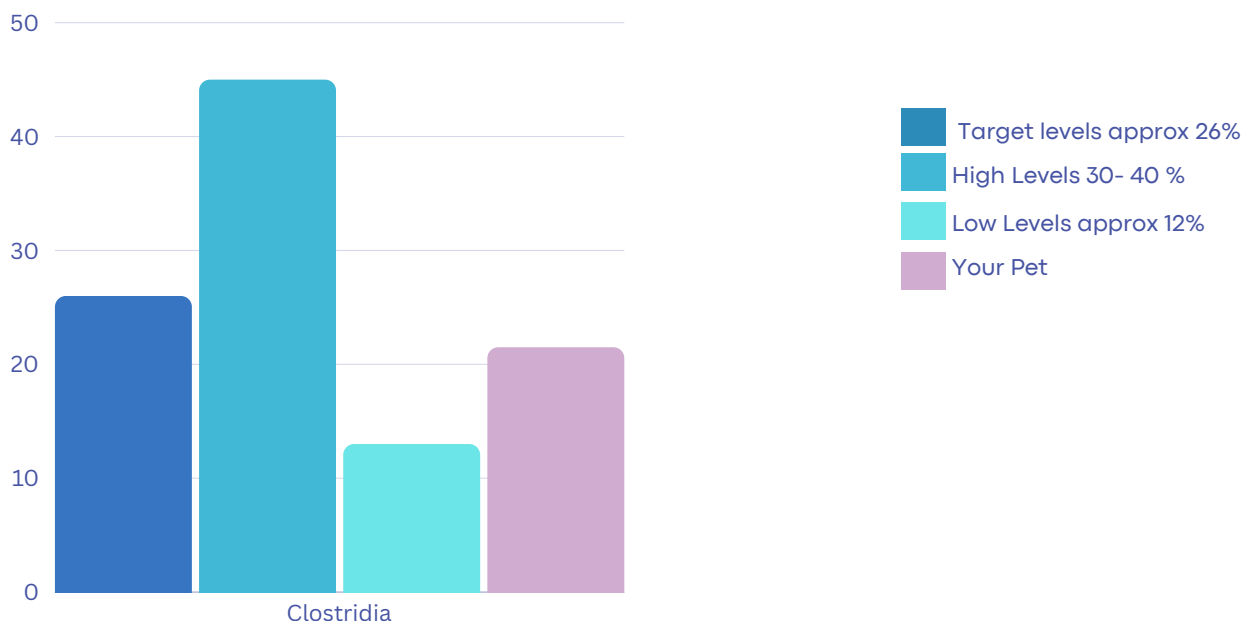
Dietary Advice

High Levels

Some members within clostridia increase in infections and illness (c. difficile, botulin) if your pet has symptoms of discomfort or infection, please check with your vet. Adding Biotic Boost to the diet should lower them.

Low Levels

Plant chemicals (antioxidants) can be used to increase the percentage of clostridia, these work by making the microbiome more favourable for clostridia members whilst reducing the bad bacteria that can make the biome more acidic and less favourable. Adding the Petbiome Prebiotic helps increase the levels of good clostridium above the bad members of the clostridia family. Rebalancing clostridia is important as your pet will have a reduced immune response and less protection against gastrointestinal discomfort and disease.



Proteobacteria

Proteobacteria are part of the core microbiome of healthy dogs, they are more abundant in dogs fed a high protein diet. Proteobacteria contain several known pathogens associated with gastrointestinal imbalances, disease, and discomfort in dogs. Including *Escherichia coli/shigella*, *Campylobacter jejuni*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, and *Yersenia enterocolitica*. Clinically healthy dogs and cats do have *Helicobacter* species though at levels of less than 0.5% of the microbiome.

Overgrowths or ‘blooms’ of proteobacteria are associated with dysbiosis, published research indicates dogs with gut inflammation and metabolic disorders do have higher levels.

If your dog has an overgrowth of any pathogenic member of the proteobacteria phyla this will be highlighted on the veterinary notes page.

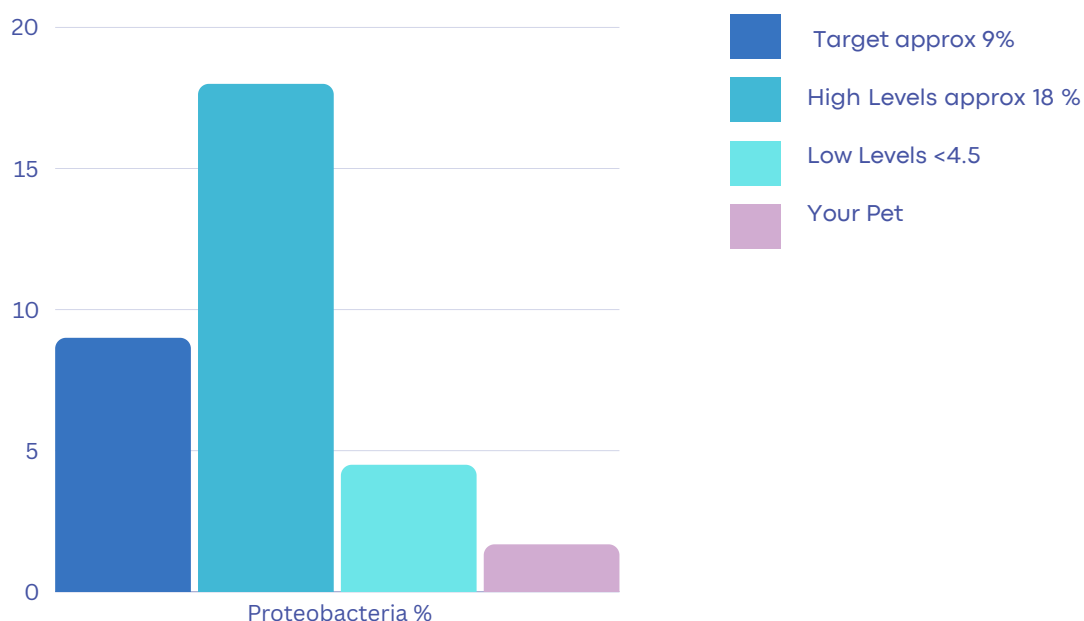
Proteobacteria are involved in the metabolism of protein and are higher in dogs fed high protein diets, and those containing highly processed ingredients, such as soya and rice meal.

Dietary Advice

If levels of proteobacteria are high check the protein content of the diet, some types of dog food can contain as high as 22% protein. To reduce proteobacteria, reduce the dietary protein content down to 8%. Levels may be high due to a pathogenic infection rather than a dietary imbalance, as explained in the first paragraph please read the Veterinary Summary on page 5. If levels are low then increasing the protein content should help.

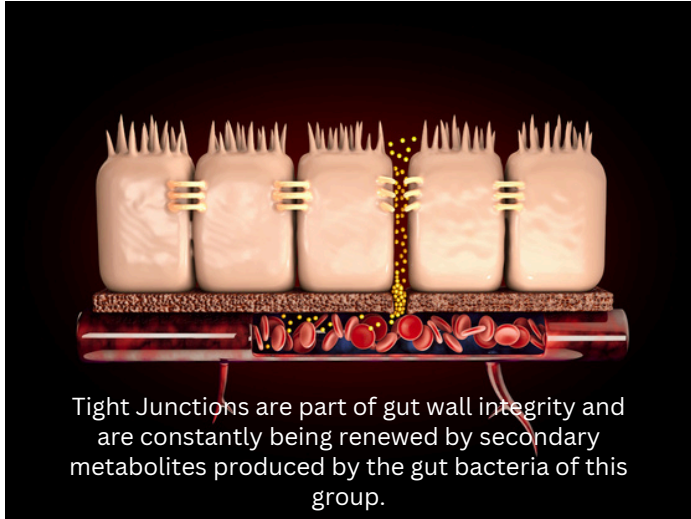
Garcia-Mazcorro, J. F., Dowd, S. E., Poulsen, J., Steiner, J. M., & Suchodolski, J. S. (2012). Abundance and short-term temporal variability of fecal microbiota in healthy dogs. *Microbiologyopen*, 1(3), 340-347.

Moon, C. D., Young, W., Maclean, P. H., Cookson, A. L., & Bermingham, E. N. (2018). Metagenomic insights into the roles of Proteobacteria in the gastrointestinal microbiomes of healthy dogs and cats. *Microbiologyopen*, 7(5), e00677.



Section 3. Direct Links Between Microbes and Health/Disease

Gut Wall Integrity and Renewal



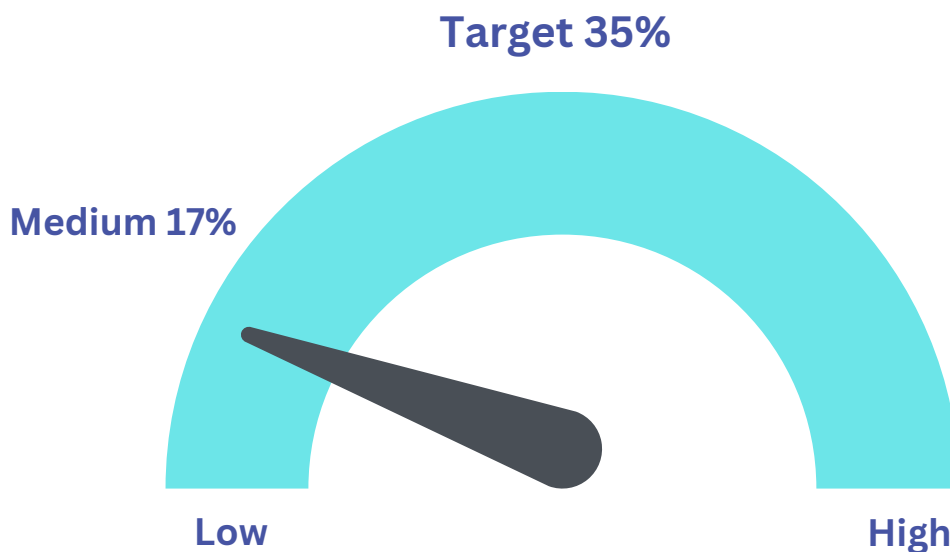
Members of this group

Lactobacillus,
Actinobacteria,
Roseburia,
Bifidobacteria

The members of this group of bacteria help to renew and maintain the tight junctions of the gut wall, a sufficiency makes the gut stronger and healthier, the target is 35%. The gut wall exists to protect the inside of the dog from any outside environmental or bacteria invaders that may cause ill health, especially pathogenic bacteria.

The gut bacteria in this group are also part of the control mechanism that exists between the gut and the brain. Low numbers of bifidobacterium and lactobacillus are directly linked to biome stress, with further links to stress related conditions such as anxiety and IBD. Many dogs are low in bifidobacterium and lactobacillus.

High Levels can be a marker for inflammation



More on the Health of the Gastrointestinal Tract

Biofilm bacteria form when there are too few of the superbugs which act as a 'police force' which protect against formation.

Biofilm Bacteria

Most bacteria reside in a fixed area as part of a biofilm community, for protection and an improved chance of survival, though biofilm formation is implicated in many chronic disease states. Biofilm bacteria commonly reside in the gut wall and aggravate/cause 'leaky gut'. Colonising gut bacteria tend to rob the host of nutrients, reduced levels of good gut bacteria linked to a strong immune response and produce toxins linked to inflammation.

Risk levels for discomfort have been calculated by taking the number biofilm bacteria and comparing this to the number of existing protecting/policing bacteria able to control and reduce numbers. A low risk indicates a high percentage of the good gut bacteria that provide protection. A high risk is an indication of too few good gut bacteria with high numbers of biofilm forming bacteria in the gut wall.

High (red) indicates a higher risk of biofilm bacteria formation with links to gastric inflammation. Low (green) indicates a lower risk of biofilm bacteria with higher numbers of good gut bacteria.



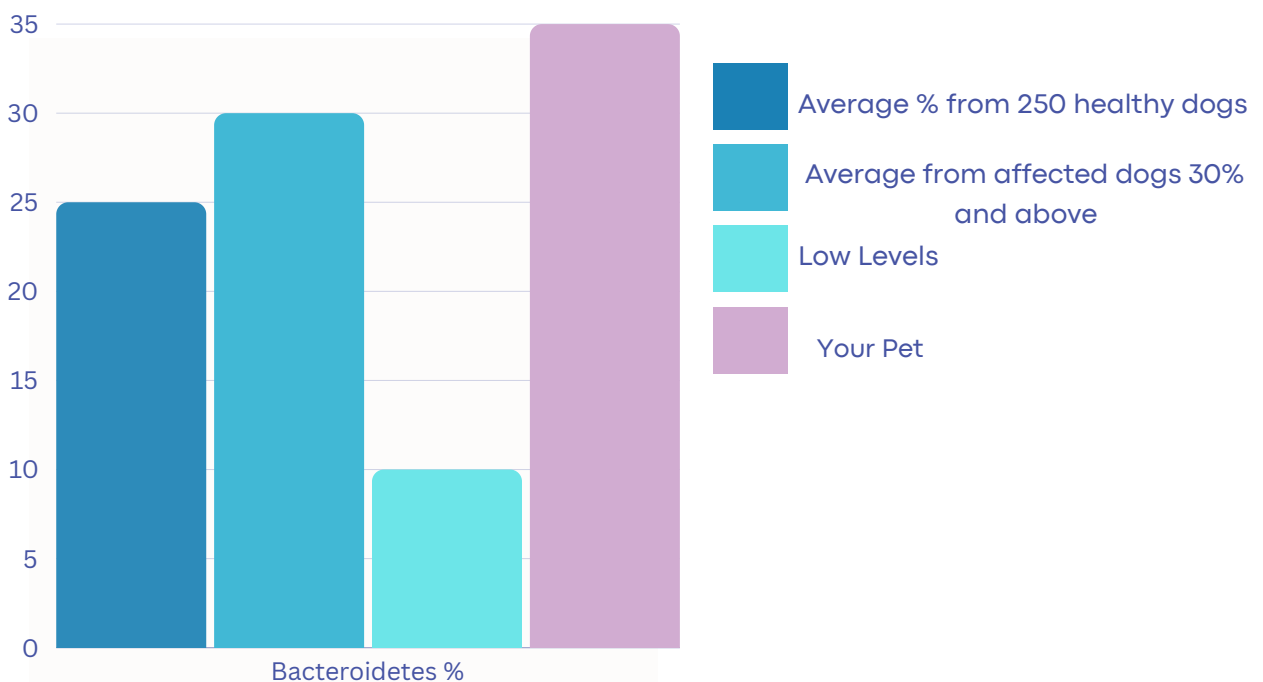
Skin Allergies

The dog has a different microbial community residing on the skin and in the ear, urinary and respiratory tracts, the different sites are ecosystems each with a common core membership. Microbes are sensitive to change and imbalances are common, causing infections and inflammation both at site and within the gastrointestinal microbial community.

Put a different way, diseases (including itching and allergies) of the skin can be linked back to gastric disturbances and imbalances through the skin/gut axis, the gut bacteria communicating through the endocrine, immune, and nervous systems.

The gut microbes are linked to many different types of inflammation including pruritis (Itching) and one of the main features is a loss of alpha diversity (species richness) especially within the phyla bacteroidetes.

The main cause for a reduction in alpha diversity is thought to be the wide use of medication such as antibiotics which reduce the numbers of predator bacteria that kill pathogenic bacteria linked to disease, such as Escherichia Coli. In the absence of predator bacteria, the pathogenic bacteria multiply and translocate across the gut wall to cause problems in other parts of the body or produce inflammation and reduced immune response. See page 6 for diversity score. As diversity decreases the microbiome becomes more favourable for the overgrowth of bacteria linked to allergies.



The risk of developing allergies is calculated from allergen linked bacteria present in the microbiome of your pet based on our own population data of dogs with allergies/itching. A low/medium diversity score (page 6) often increases the risk of developing allergies.

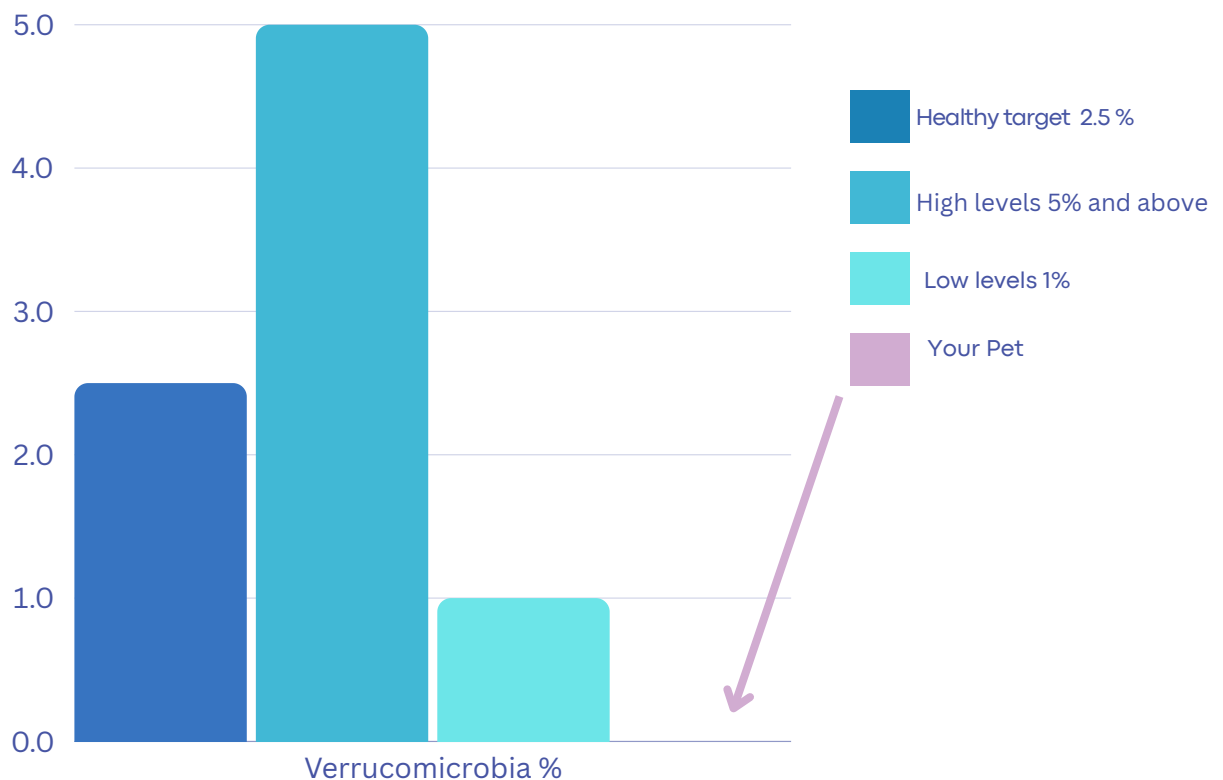
Metabolism

Verrucomicrobia Akkermansia levels relate to efficient glucose metabolism and has been calculated (from our own population data) to be 2.5- 3%, low levels indicate a sluggish/poor glucose metabolism in the gut. Low levels of Verrucomicrobia Methyacidaphales relate to a reduced insulin/GLP -1 function. Akkermansia is considered to be a good measure of a healthy biome it is directly linked to insulin sensitivity. It is also an important anti-inflammatory, helps repair the gut wall, and has a direct relationship with the immune system.

Low levels of V. Akkermansia are common and may contribute to ill health associated with metabolism including weight gain/obesity.

Gut bacteria can play a role in preventing weight gain through the digestion of food and selection and absorption of nutrients, one of the main components of weight gain is a loss of biodiversity and a reduction of the number of species associated with leanness.

Following several years of research into the microbial communities living within the gastrointestinal tracts of overweight/obese dogs, the PetBiome Prebiotic Plus contains the nutrients and active plant compounds that help select and feed the bacteria within this group.

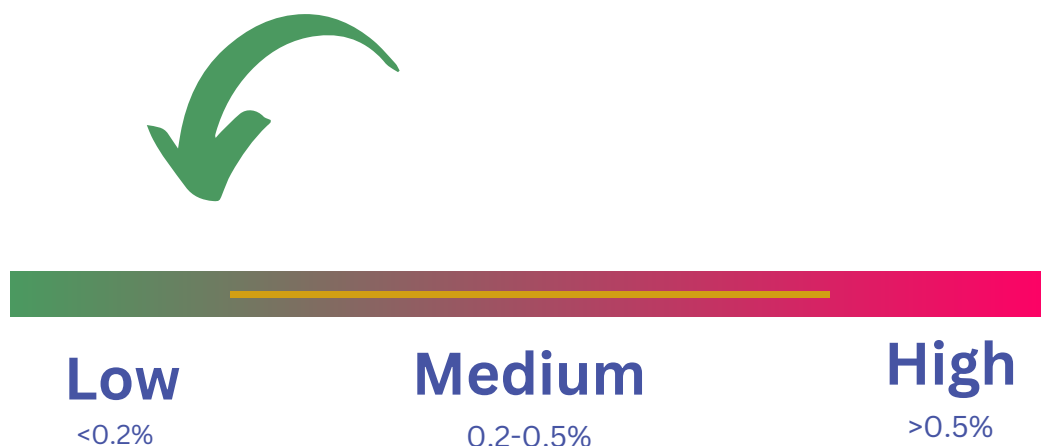


Bloating

Methanogens that produce methane gas are not “bacteria” but belong to the Archaea kingdom.

Syntrophy is a term used to describe the relationship between two bacteria that help to feed each other and maintain a favourable environment to ensure each other’s survival. Methanogen archaea have a syntrophic relationship with other microbiota, converting CO₂ and H₂ to CH₄, they also produce short-chain fatty acids (SCFAs) from carbohydrate, which contributing to 10% of the host’s daily energy requirements. One of the main roles of methanogens is to maintain the hydrogen gas balance in the gut, alterations (too many or too few) may increase the risk of gut dysbiosis/disease.

Your Dogs Methanogen Score



Happy Chemicals

Dogs can be aggressive for different reasons, aggression being categorised in relation to dominance, fear, food and territorial.

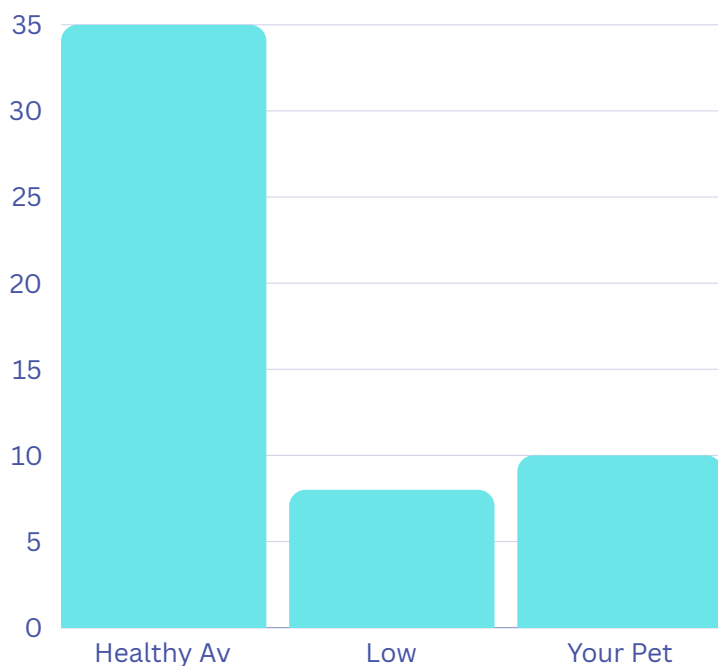
There is also a 5th category, recently identified by science and linked to the microbiome of the dog which may be more common and relates to gut microbiome imbalances (called dysbiosis), this type of imbalance can also include anxious (phobic) behaviour.

Here's how it works.

The gut bacteria communicate with the brain by making chemicals that send signals or cause reactions in the nerves and hormones. This connection is called the gut/brain axis. The brain uses 3 key chemicals in relation to mood, a high proportion of all three are produced solely by the gut bacteria, mood changes are therefore easily influenced or changed by the members of the microbial community.

- Serotonin, seventy percent of the 'happy chemical' is produced in the gut affects mood and anxiety levels.
- Dopamine, fifty percent is manufactured in the gut.
- GABA, microbes use putrescine and glutamate to produce GABA which regulates stress and anxiety.

Full metagenomic sequencing is the best way to analyse whether the gut is producing enough of the 'happy chemicals', using an algorithm to calculate levels of chemicals being produced.

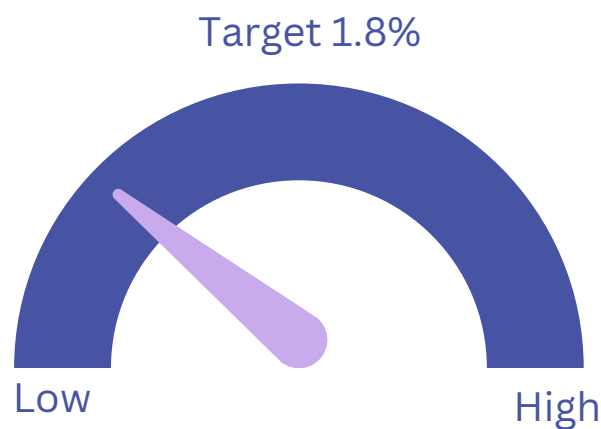


In a healthy microbiome 35% of the inhabitants would be involved in the production of happy chemicals. If your pet is too high or too low the Petbiome Prebiotic Plus will help to restore balance.

Temperament

Anxious Phylogenetic Type

Both of these bacteria are linked to good health and temperament, low levels are indicative of an anxious phylogenetic type. It is possible to supplement with a live probiotic for both species (rhamnosus and bacilli) or alternatively consider a faecal transplant from a healthy/calm donor.

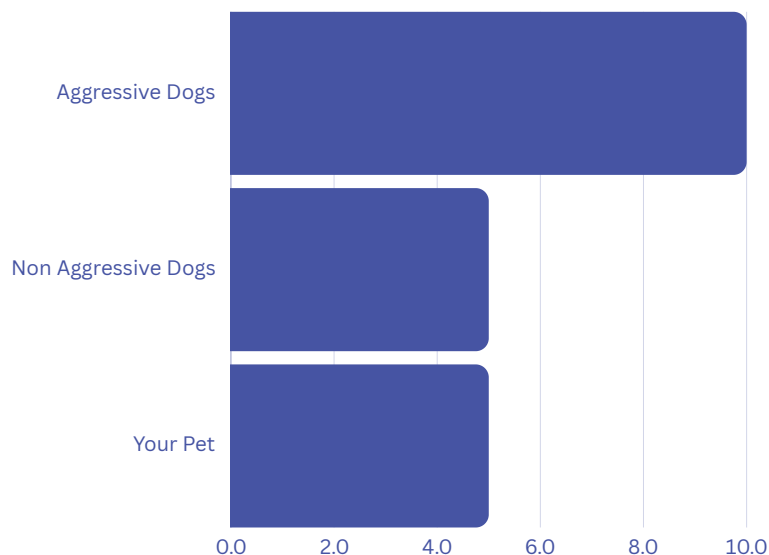


Aggressive Phylogenetic Type

Higher percentages of the bacteria within this group (Lachnospiraceae, Erysipelotrichaceae and Clostridiaceae) at 10% or higher are indicative of greater aggression.

Kirchoff, N. S., Udell, M. A., & Sharpton, T. J. (2019). The gut microbiome correlates with conspecific aggression in a small population of rescued dogs (*Canis familiaris*). *PeerJ*, 7, e6103. Mondo, E., Barone, M., Soverini, M., D'Amico, F., Cocchi, M., Petrulli, C., ... & Accorsi, P. A. (2020). Gut microbiome structure and adrenocortical activity in dogs with aggressive and phobic behavioral disorders. *Heliyon*, 6(1), e03311

Mondo, E., Barone, M., Soverini, M., D'Amico, F., Cocchi, M., Petrulli, C., ... & Accorsi, P. A. (2020). Gut microbiome structure and adrenocortical activity in dogs with aggressive and phobic behavioral disorders. *Heliyon*, 6(1), e03311



Environmental Pollutants and the Microbiome

Glyphosate-based herbicides (GBHs) are the most frequently used herbicides globally. Residues from the use of GBH's have a profound effect on the microbiome of animals and humans, reducing the ability of the microbiome to protect the host against free radical damage and increasing oxidative stress.

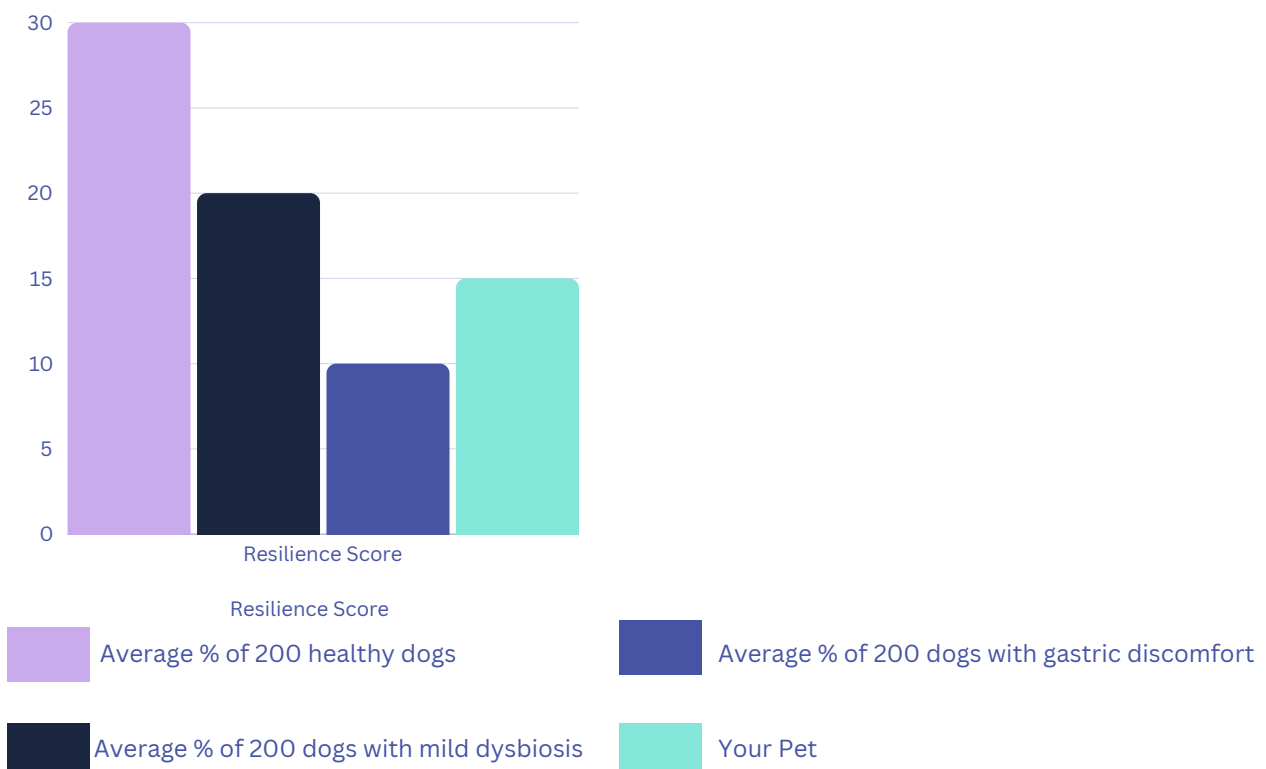
The microbiome exposed to GBH residue becomes a more favourable environment for pathogenic bacteria to thrive.

Resilience to the effects of GBH is calculated by the percentages of environmental pathogens and those bacteria that produce antioxidants that reduce oxidative stress, from the phyla firmicutes and including important members of lactobacillus.

How resilient is your pets microbiome to GBH

If your reading is low this means your dog has a experienced higher exposure to GBH's and has a lowered ability to protect against the effects of oxidative stress and free radical damage. Increasing dietary plant polyphenols will help to increase the existing low levels of good gut bacteria whilst reducing pathogens such as cyanobacteria and shewanella.

If your reading is high then this means your dog has had a lower exposure to GBH's or has a higher resilience to it's effects, a higher score is obviously preferable.



Thank You For Using PetBiome

We really hope you have found this report a useful tool to support the good health of your dog throughout all the stages of his life. If you would like to discuss this report in greater detail we are able to help. We offer a free 15 minute session to go through your report (please phone or email to book). For longer sessions relating to helth/diet/discomfort/ disease please book a longer session through the PetBiome shop (£45).

Please let us have feedback or contact us if you need any further info on any topic within this report.



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