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Basepaws Feline Dental Health Test

Contents:

The feline oral microbiome

Environmental factors and various food sources make the feline oral cavity a place of unique interactions between microbes (the oral microbiome) and the feline host.

The almost constant exposure to foreign microbial organisms has made the oral microbiome fiercely competitive. It is populated by microbes which are good at defending their territory and are mostly able to avoid being replaced by foreign invaders, including pathogens. However, once in a while, pathogenic microbes manage to colonize disproportionately large parts of the oral cavity which can be associated with pathology. The state of the oral microbiome can reveal information about the health of tissues in the mouth and point to potential dental and gum diseases[1]. It is now well established that most dental diseases are caused by a complex interaction of multiple microbes, as opposed to having a single microbial culprit[2].

The field of oral microbiome research in companion animals is still in its infancy, with existing studies having small sample sizes (fewer than 100 animals) and focusing mainly on periodontal disease. However, there is evidence that the oral microbiome profiles of humans, cats and dogs share some noteworthy similarities[1,3,4]. This suggests that existing studies in humans can help us better understand dental disease-relevant microbial changes in the feline oral microbiome. There is also evidence that surveying the human oral microbiome via buccal, supragingival or subgingival sample collection method, can serve as an early indicator of dental disease-associated processes not yet visible to the naked eye[5].

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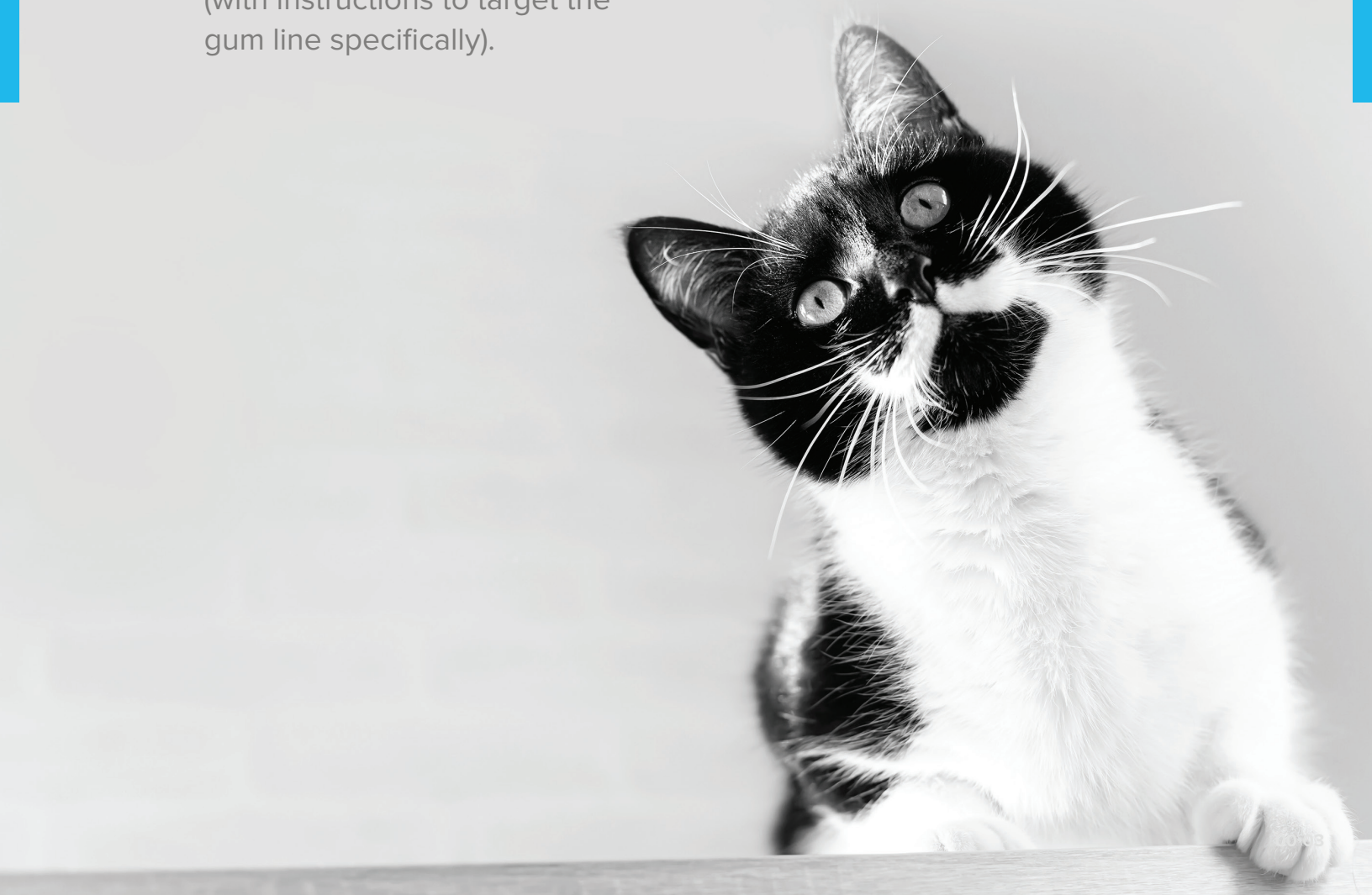
Basepaws' Dental Health Test

The Basepaws Dental Health test provides an easy way for pet owners to test their cat's oral microbiome and spot any signs of microbial dysbiosis associated with disease. Sample collection can be done at home by the pet owner or by a veterinarian at the clinic. A buccal swab is used for sample collection (with instructions to target the gum line specifically).

Our test currently focuses on detecting oral microbiome signatures characteristic of feline periodontal disease, tooth resorption and bad breath (halitosis). The aim of our test is to facilitate early detection of dental problems. Future iterations of this test will include detection of oral microbiome patterns associated with gingivostomatitis, as well as other dental and systemic diseases.



Basepaws' shotgun metagenomic sequencing approach

In the last decades of the 20th century, the characterization of the microbiome was limited to the identification of bacteria that could be cultured in the lab. It is estimated that fewer than 2% of all existing bacteria are culturable[6], meaning that studies relying on this method suffer from a significant underrepresentation bias. Nowadays, studying the microbiome has advanced significantly, with the help of Next Generation Sequencing (NGS) which does not rely on bacterial culturing. Most currently available direct-to-consumer microbiome tests use a technique called '16S rRNA gene sequencing'. While this technique provides substantially more information than early bacterial culturing efforts, it can only be used for identifying bacterial species (and some archaea) present in the microbiome. However, it is well-known that the microbiomes of different sites of the body can be composed of viruses, protozoa, and fungal species, in addition to bacteria and archaea. This means that the 16S rRNA gene sequencing approach zooms in on just one part of the microbiome, ignoring the rest. Additionally, it does not provide sufficient resolution to reliably and consistently go beyond the genus level of taxonomic classification. Therefore, in most cases, we do not know the exact species of bacteria comprising the microbiome, making data-driven conclusions vague and relying on approximation.

To address these problems, Basepaws uses shotgun metagenomic sequencing instead of 16S rRNA gene sequencing. This method allows us to capture complete genomes of organisms across all domains of life (not just the 16S region of the genome), not restricting us to only bacteria. In addition, we can reliably identify organisms to the species or even strain level, making our analysis more accurate and definitive. Lastly, while the 16S-based approach yields hundreds of taxonomically classified bacteria, our shotgun metagenomic approach can, on average, allow us to identify over 1,000 microbial species in the mouth of a cat. In our database of 35,000 feline oral microbiomes, we identified ~9,000 microbial species. We observed that the average feline oral microbiome is composed of 97.5% bacteria and archaea, 0.27% DNA viruses (RNA viruses cannot be detected with shotgun metagenomic sequencing), 0.02% phages and <2% fungi. These numbers paint a richer, more accurate picture of the feline oral microbiome, compared to culture-dependent and 16S-based sequencing approaches.

Analytical approach, accuracy and validation

Dental health assessment

For our analysis, we took the sequencing reads from every oral microbiome sample in our database that was processed through a ligation-based sequencing library preparation method and had accompanying user-provided dental health history data. We classified all of these samples' sequencing reads using the KRAKEN2 metagenomic sequence classifier to identify all present microbial organisms[7]. The recommended confidence score of 0.1 was used as a cutoff for the KRAKEN2 classification algorithm. We removed all samples with fewer than 10,000 classified microbial reads and more than 500,000 classified microbial reads. Next, we removed all microbial species with a non-zero mean of fewer than 10 reads. We then used Bracken[8], a statistical method for calculating abundance of species in DNA sequences from a metagenomic sample.

After we filtered the data in the manner explained above, we focused our attention specifically on 5 groups of samples:

- Cats reported by their owners to have been diagnosed by a veterinarian with **p**eriodontal **d**isease (**PD cohort**) – 441 cats.
- Cats reported by their owners to have been diagnosed by a veterinarian with **t**ooth **r**esorption (**TR cohort**) – 77 cats.
- Cats reported by their owners to have **b**ad **b**reath (**BB cohort**), also characterized as 'death and decay' breath – 133 cats.
- Cats 1-3 years of age, with no diagnosed dental or general health conditions (**Healthy cohort**) – 848 cats. Cats below one year of age were excluded from this group in order to not bias the healthy cohort results to the specific composition of the kitten oral microbiome. Since age is a known predictive factor for dental and general diseases, the 1-3 age range was selected for this cohort in order to minimize the possibility that older cats with yet undiagnosed diseases could be misclassified as healthy cats.
- Cats reported by their owners to have '**t**ypical' cat **b**reath (**TB cohort**) – 3,072 cats.

Cross-validation with available literature

Before going any further with our analysis, we wanted to make sure that the patterns we see in our data are consistent with published research. The majority of the otherwise scarce feline oral microbiome scientific literature focuses on periodontal disease. Some of the key microbial characteristics of periodontal disease across cats (and humans) described in literature include **increased abundance** of:

- Bacterial species **Porphyromonas gingivalis**[3]
- Bacterial species **Tannerella forsythia**[9]
- Bacterial species **Bacteroides zoogleoformans**[10]
- Bacterial species **Desulfomicrobium orale**[11]
- Bacterial species **Desulfovibrio fairfieldensis**[12]
- Bacterial species **Treponema denticola**[9]

Conversely, **decreased abundance** of the following microbes is also observed in periodontal disease in cats:

- Bacterial genus **Moraxella**[2]
- Bacterial genus **Capnocytophaga**[2]

Our analysis detected a multitude of microbes significantly upregulated or downregulated in periodontal disease in cats. In addition to a plethora of newly identified microbes playing a role in the dysbiosis associated with the disease, we also observed the patterns characteristic of periodontal disease that had been previously described in literature (**Table 1**).

Table 1. Selected microbial species which show significantly increased or decreased abundance in periodontal disease compared to control ($p<0.05$).

The average percentage increased or decreased abundance for each microbial species when compared to a healthy control (calculated using a centered log-ratio transformation) is shown in pink and blue, respectively. Microbial species previously described in scientific literature as misregulated in periodontal disease are shown in bold font.

Microbes with increased abundance in PD cohort	% increase compared to Healthy cohort	Microbes with decreased abundance in PD cohort	% increase compared to Healthy cohort
Bacteroides sp. HF-5287	+49%	Frederiksenia canicola	-47%
Bacteroides zoogleoformans	+47%	**Moraxella bovis**	-33%
Bacteroides sp. M10	+41%	Mannheimia haemolytica	-32%
Odoribacter splanchnicus	+38%	Pseudoleptotrichia goodfellowii	-32%
Desulfobulbus oralis	+36%	Streptobacillus moniliformis	-32%
Bacteroides caccae	+35%	**Capnocytophaga sp. H4358**	-29%
Desulfomicrobium orale	+35%	**Capnocytophaga sp. H2931**	-29%
Bacteroides sp. CBA7301	+33%	**Moraxella catarrhalis**	-28%
Bacteroides uniformis	+33%	Alysiella filiformis	-28%
Parabacteroides distasonis	+33%	**Moraxella cuniculi**	-27%
Bacteroides ovatus	+32%	**Moraxella ovis**	-27%
Bacteroides caecimuris	+32%	**Moraxella bovoculi**	-27%
Desulfovibrio fairfieldensis	+26%	Neisseria zoodegmatis	-27%
Porphyromonas gingivalis	+26%	Neisseria weaveri	-26%
Bacteroides heparinolyticus	+25%	**Capnocytophaga cynodegmi**	-25%

Microbes with increased abundance in PD cohort	% increase compared to Healthy cohort	Microbes with decreased abundance in PD cohort	% increase compared to Healthy cohort
Actinomyces sp. Chiba101	+25%	Neisseria animaloris	-25%
Bacteroides thetaiotaomicron	+25%	Cutibacterium acnes	-24%
Paraprevotella xylaniphila	+25%	Neisseria chenwenguii	-23%
Actinomyces howellii	+25%	Neisseria elongata	-22%
Bacteroides xylanisolvens	+24%	Neisseria dentiae	-22%
Bacteroides helcogenes	+24%	Kingella oralis	-22%
Petrimonas mucosa	+24%	Neisseria canis	-22%
Desulfovibrio desulfuricans	+24%	Pelistega sp. NLN63	-21%
Bacteroides fragilis	+23%	Neisseria wadsworthii	-21%
Bacteroides sp. A1C1	+22%	**Moraxella osloensis**	-21%
Treponema sp. OMZ 838	+22%	**Capnocytophaga canimorsus**	-21%
Proteiniphilum saccharofermentans	+22%	Epilithonimonas vandammei	-19%
Treponema brennaborense	+22%	Lysobacter oculi	-19%
Treponema putidum	+22%	Streptococcus dysgalactiae	-18%
Treponema denticola	+21%	Riemerella anatipestifer	-18%
Treponema pedis	+11%	**Capnocytophaga stomatis**	-17%
Acidovorax monticola	+11%	Fusobacterium hwasookii	-17%
Propionibacterium freudenreichii	+11%	Cardiobacterium hominis	-17%

Microbes with increased abundance in PD cohort	% increase compared to Healthy cohort	Microbes with decreased abundance in PD cohort	% increase compared to Healthy cohort
Treponema phagedenis	+11%	Acinetobacter johnsonii	-17%
Prevotella denticola	+10%	Neisseria shayeganii	-16%
Acidovorax sp. RAC01	+10%	Fusobacterium pseudoperiodonticum	-16%
Tannerella forsythia	+10%	**Pasteurella multocida**	-16%

Risk assessment for periodontal disease, tooth resorption, and bad breath

Having validated our findings with existing literature, we built a pipeline for classifying cats' risk level for developing (or already having) periodontal disease, tooth resorption and bad breath. In short, our pipeline compares the microbial composition of each feline oral microbiome sample to the microbial composition of samples from known 'healthy' cats and cats known to suffer from each of the three conditions. Our pipeline then generates a probability score used to assess whether the queried feline oral microbiome sample belongs to a cat that suffers from one of the three conditions.

First, we used Pairwise Log-Ratio (PLR) transformation[13] of the sequencing reads for each feline microbiome sample to account for potential data compositional biases, which are a well-known problem in microbiome studies[14]. Then, we found the most significant PLRs (p-value < 0.01) between control and condition by performing a z-test. We performed the following comparisons between condition and control:

- PD cohort vs Healthy cohort
- TR cohort vs Healthy cohort
- BB cohort vs TB cohort

Next, we counted the frequency of microbial species for all significant PLRs. We kept all species where the frequency was 50% or more of the maximum possible comparisons for that species. We called these microbial species 'predictive' for each respective dental condition. For periodontal disease, we identified 110 predictive microbes, for tooth resorption – 70 predictive microbes, and for bad breath – 138 predictive microbes.

For each of our three dental conditions of interest, we scored each sample by comparing the predictive pairwise log-ratios (pPLRs) of the sample to the mean pPLRs of controls, taking into account the direction and magnitude of the difference.

We plotted the distribution of these scores for every condition as it compares to its respective control cohort (**Figure 1**). Despite our best effort to minimize the chances of including undiagnosed cats suffering from a dental condition in our healthy cohort, it does seem to be the case that a small proportion of cats reported by their owners to be healthy, do in fact show some patterns consistent with dental disease or bad breath. The most likely reason for this is that, at the time of reporting, the pet owner was not aware of the developing dental condition in their cat.

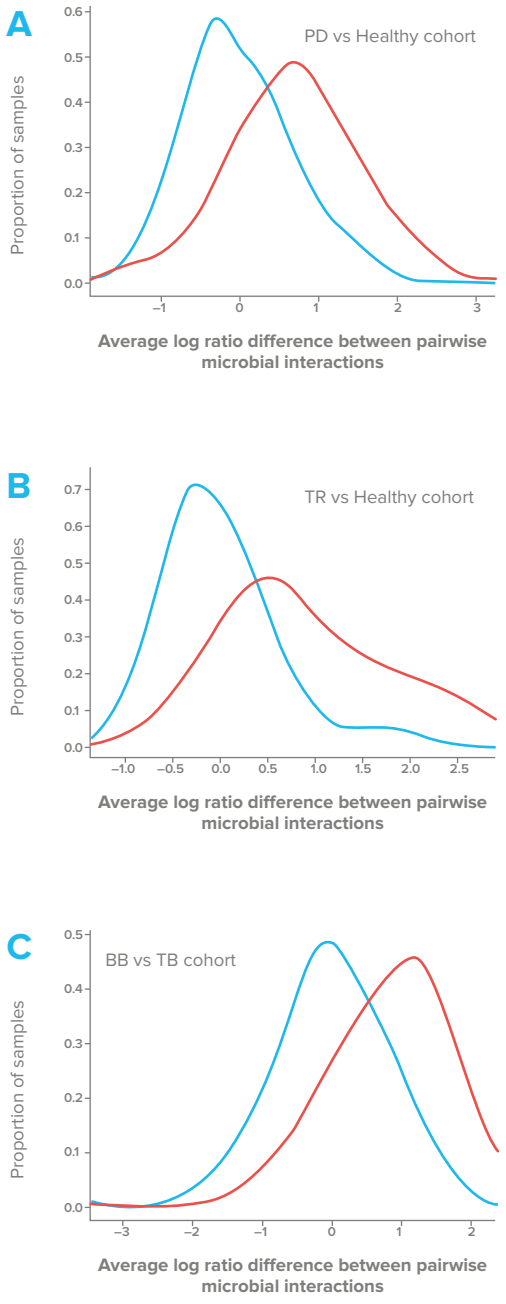


Figure 1. Distribution of average log ratio difference score between pairwise microbial interactions associated with (A) PD and healthy cohorts, (B) TR and healthy cohorts, and (C) BB and TB cohorts.

We fitted 3 Gaussian mixture models (one for each dental condition of interest) with 2 components each - healthy cohort and dental condition - onto the distribution of the average log ratio difference score between pairwise microbial interactions. This modeling approach allowed us to generate a score for each sample reflecting its probability of belonging to the control cohort or the respective dental condition cohort. We plotted this probability distribution for each sample belonging to each condition (**Figure 2**).

We set probability risk classification ranges for each condition as follows:

Probability score:
0.00 - 0.33 - '**LOW RISK**' for having the condition

Probability score:
0.34 - 0.66 - '**MEDIUM RISK**' for having the condition

Probability score:
0.67 - 1.00 - '**HIGH RISK**' for having the condition

We tested the sensitivity (ability to detect cats known to suffer from a dental condition) and specificity (ability to detect cats in the control cohort as **not suffering** from a dental condition) of our risk classification method for each condition. Results are summarised in **Table 2**.

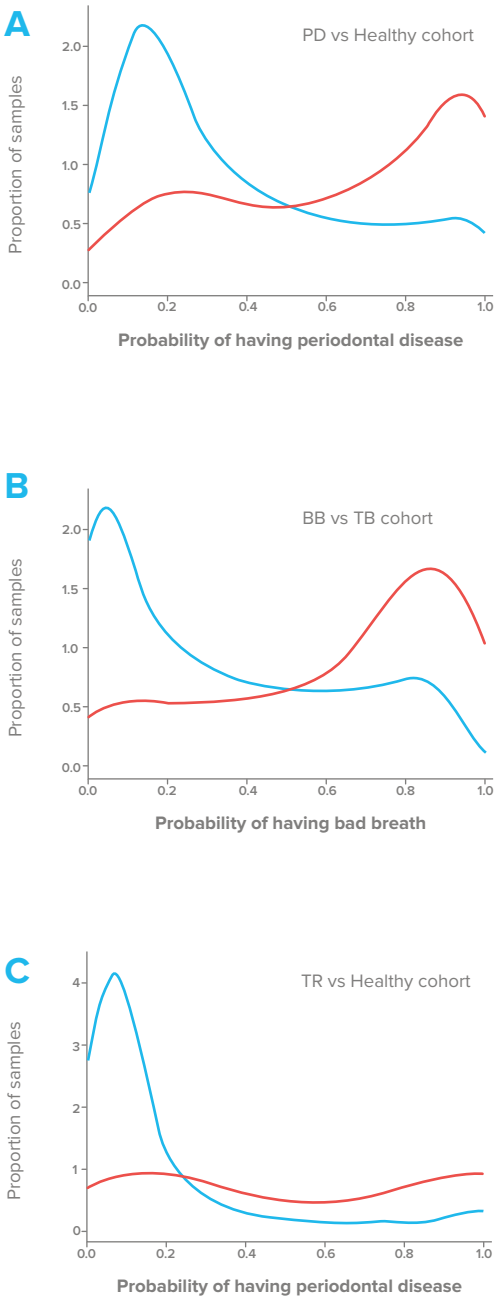


Figure 2. Distribution of the probability of having (A) periodontal disease, (B) tooth resorption, and (C) bad breath based on a 2-component Gaussian mixture model.

Table 2. Sensitivity and specificity of our 2-component Gaussian mixture model for dental condition risk assessment.

Condition	**Sensitivity** (% known positive samples classified as medium/high risk)	**Specificity** (% known negative samples classified as low risk)
Periodontal disease	76%	57%
Tooth resorption	59%	85%
Bad breath (halitosis)	81%	58%

Our model has the lowest sensitivity for tooth resorption. One potential explanation for this observation is related to the nature of the pathology behind tooth resorption. This condition originates inside of the tooth and, as it enters more advanced stages, reaches the surface of the tooth. It could be the case that the microbes associated with tooth resorption can best be detected when the lesion has reached the surface of the tooth. This suggests that, in some cases, it is possible that a diagnosis of tooth resorption could be made before the microbial signature of tooth resorption is reliably picked up by a microbiome test.

Our model also has relatively lower specificity for periodontal disease and bad breath. One interpretation of this result is the possibility that our healthy and TB cohorts might include some cats with periodontal disease or bad breath, respectively, that have not yet been diagnosed by a veterinarian or noticed by the pet owner.

Detailed microbial breakdown assessment for each dental condition

Our Dental Health report has a section focused on the top 3 pathogenic microbes for periodontal disease, tooth resorption and bad breath. To generate these results, from the previously identified predictive microbes for each dental condition, we identify the top 3 microbes contributing the most to the risk of developing each of the three conditions. In other words, for each sample, we select the three microbes that have the largest average magnitude of difference relative to other microbes in the group when compared to control.

From the same set of predictive microbes for each dental condition, for every sample, we isolate the top 10 microbes which have the highest number of significant interactions with other predictive microbes for this condition. Next, we normalize the number of these interactions for each of the top 10 predictive microbes to a score from 1 to 5. In this scoring system, for each of the top 10 microbes, 1 denotes the lowest probability that this particular microbe is contributing to the dental condition of interest. In contrast, a score of 5 denotes the highest probability that this microbe is contributing to the dental condition of interest (**Figure 3**).

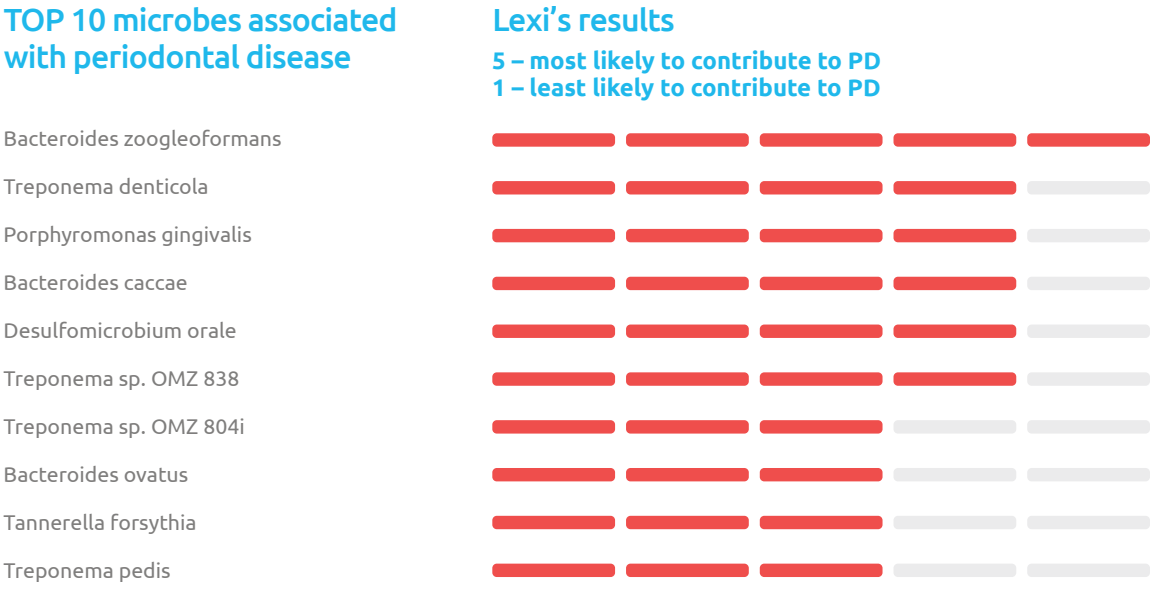


Figure 3. Example Basepaws Dental Health report section focusing on the top 10 microbes associated with periodontal disease in a cat named Lexi.

Detection of trace amounts of DNA from non-microbial species

Apart from the thousands of microbial species we detect in each feline oral microbiome sample, we are also able to detect DNA from non-microbial organisms (plants and animals). The presence of trace amounts of DNA from such organisms is, most likely, linked to the type of food(s) and supplements consumed by each cat.

To classify the non-microbial and non-feline DNA present in each cat's mouth, we use KRAKEN2 on a custom-made database containing every representative complete plant genome available on NCBI, as well as complete genomes representative of each major animal group. For each cat, we report the top five plants or animals with the highest number of sequencing reads.

Conclusions

Basepaws developed the first of its kind at-home feline dental health test based on an assessment of the oral microbiome. With each test, we can identify >1,000 microbial species per sample. Our large oral microbiome reference database allows us to identify a multitude of novel associations between microbes found in the mouth and a variety of diseases, as well as confirm previously reported findings. However, the field of feline oral microbiome science is extremely young and understudied, which is why we report only on conditions and microbes where previous knowledge exists and/or we see a particularly strong signal coming through in our data.

As we accumulate more data and conduct more controlled studies and analyses, we will aim to continuously enrich this report, improve its predictive power, and provide even more helpful insights. We want to emphasize that the identification of a certain microbial signature associated with a dental disease does not constitute a diagnosis. Conversely, not detecting a particular microbial signature does not exclude the possibility of an unknown disease-causing pathogen being present or dental disease being caused by something other than pathogenic microbes. This report does not aim to substitute a diagnosis by a professional.

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Thank you.