Human-feline oral microbiome cross-species transmission and its association with idiopathic tooth resorption.

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Abstract

Tooth resorption is an uncommon condition which is irreversible and highly destructive. Once detected and diagnosed, treatment options are limited because of the destructive nature of the resorptive lesions. Unlike dental caries and periodontal disease, the etiology, including pathogenic bacteria involved, of resorptive tooth lesions is not known and the causes are possibly multifactorial. Cats are reservoirs of disease and have the potential to transmit disease directly or indirectly to humans who share or have been exposed to the same environment as the animal. One Health is the concept that human health, physical and mental health, is interconnected with animal health and the health of our environment. Based on clinical observation we speculate that contact with cats and the transmission of oral bacteria from cats to humans may be linked to tooth resorption in humans. **Objective:** The purpose of this study was to investigate the oral microbiome of humans with tooth resorption and compare their oral microbiome to 1. humans without resorptive lesions and 2. the oral microbiome of cats and to investigate the potential environmental factors associated with humans with tooth resorption, in particular contact with cats. **Methods:** oral plaque samples were collected from 10 human participants with tooth resorption and 10 matched sample controls for age and sex were collected from humans without tooth resorption. Each participant completed a questionnaire. All plaque samples were processed for taxonomic assignment through DNA extraction, PCR and sequencing. Samples were statistically analyzed through taxonomic composition, alpha diversity, beta diversity and differential abundance testing with the results being compared to the feline oral microbiome. Descriptive analysis was performed on questionnaire data. **Conclusion:** There is not a statistically significant difference between the oral microbiome of humans with and without tooth resorption. Humans and felines share a similar oral microbiome overall. However, there are

three bacterial genera (*Alysiella*, *Prevotella* and *Rothia*) that appear to be in common between the human oral microbiome and the oral microbiome of felines with resorptive lesions. There is no statistically significant association between tooth resorption and contact with cats based on descriptive data.

Keywords: external tooth resorption, internal tooth resorption, oral microbiome, feline oral microbiome, cats

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This research would not have been possible without my advisor Dr. Kangmin Duan and my committee members Dr. James Elliott Scott and Dr. Eraldo Batista. Many thanks to my colleagues at the School of Dental Hygiene, dental specialists, dentists, and dental hygienists who supported my research efforts. Thank you to all students, staff, and faculty members I have studied and worked with in the department of Oral Biology – your kindness, assistance and support have made this project possible.

Dedication

My efforts to complete my thesis would not have been possible without the support from my family and friends. I dedicate this to my husband Robert who has made many sacrifices to support me through my studies and has kept me grounded through this process, my children Bruno and Beatrice who inspire me to be a better person, my parents Jim and Eleanor who raised me to never stop believing in myself, to my sister Christina for always encouraging me and my friends who support me mentally, emotionally, and spiritually every day. Maarsii.

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Chapter 1. Introduction

Oral disease, in the form of cavities and periodontal disease, has been studied extensively. The risk factors, risk indicators, etiology, prevention, treatment, and pathogenic bacteria associated with cavities and periodontal disease are well known. Many options are available to prevent and arrest decay and prevent and treat periodontal disease before tooth loss occurs. Much less is known about the etiology and prevention of another type of highly destructive, irreversible oral disease known as tooth resorption (1–11). Tooth resorption is an uncommon, not well understood condition with an estimated prevalence of 0.1%-10% (1–11). The pathogenic bacteria associated with resorptive lesions are unknown. Once tooth resorption is detected and diagnosed, treatment options are limited because most of the tooth destruction occurs before clinical or radiographic signs are visible (1–11). Often times tooth extraction is the only option leaving the patient seeking tooth replacement options (1–11).

The potential causes of resorptive tooth lesions are multifactorial and may include: history of trauma, orthodontic treatment, endodontic treatment, intracoronal bleaching, viral infection, genetics and idiopathic including contact with cats (12). It is not clear how and why these lesions start in patients with tooth resorption; a knowledge gap exists in this area of oral health.

Tooth resorptive lesions are sometimes mistaken for cavities, but they are distinguishingly different. Cavities are the result of passive inorganic demineralization of enamel initiated by known cariogenic pathogens (13). Resorptive lesions occur through active progressive destruction of dental tissue cells by clastic cells (odontoclasts) eventually resulting in granulation tissue (2,9). Tooth resorptive lesions are further classified into external and internal

resorptive lesions (2,9). External resorptive lesions (ERL) occur on the external surface of the root – the periodontium (2). Internal resorptive lesions (IRL) occur on the wall of the root canal, extend outward (pupal into dentin) and are usually a consequence of pupal inflammation (9). The tooth may appear pink in its advanced stages due to the accumulation of granulation tissue in the coronal dentin (14). Clinical detection of ERL and IRL is challenging because they can mimic either the appearance of a cavity or present as a very small perforation in the crown of the tooth (2,9). Radiographs are needed to properly and accurately diagnose resorptive lesions (2,9). Figure 1 shows ERL clinically and radiographically (12) and Figure 2 shows IRL clinically (14) and radiographically (15).

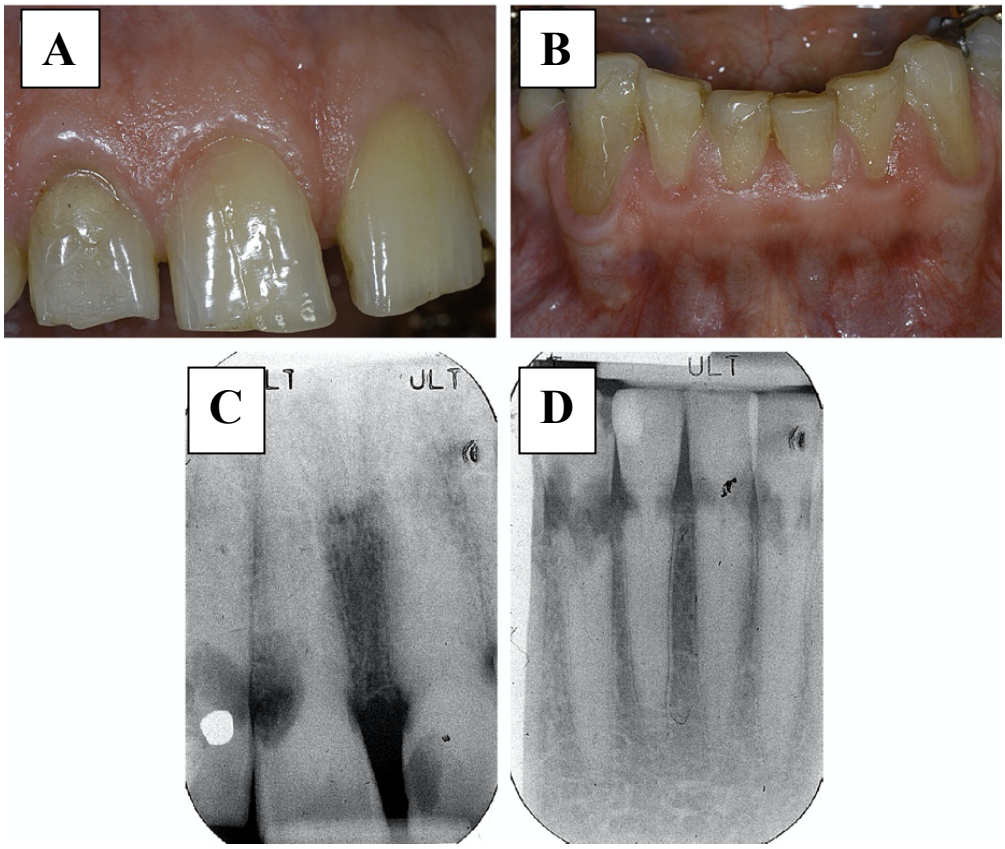

Figure 1: *A & B:* Clinical presentation of ERL. *C & D:* Radiographic presentation of ERL (12).

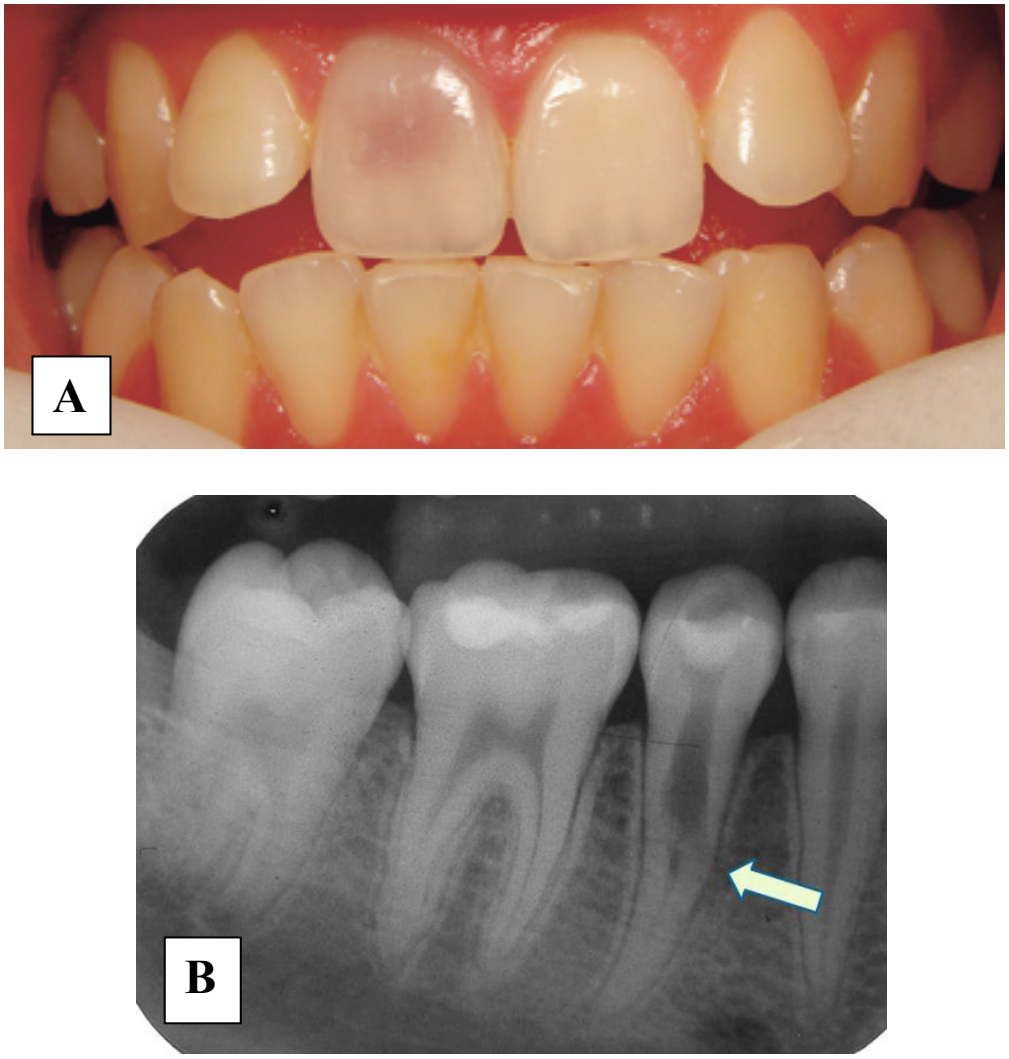

Figure 2: *A:* Clinical presentation of IRL (14). *B:* Radiographic presentation of IRL (15). Figure 2B has been used with permission under CC BY-NC-ND 4.0

One of the potential causative factors of resorptive lesion may be the microbiota associated with it. Understanding the oral microbiota of humans with tooth resorptive lesions and the possible pathogens associated with resorptive tooth lesions could provide better

understanding the etiology of these lesions and potentially aide clinical detection of the lesions through oral microbial analysis.

The literature speculates that a connection exists between humans with resorptive lesions and their environment, specifically contact with cats (12). In 2016, 41% of all Canadian households had at least one cat, a number that has risen over the last ten years (16). Given the number of people who are cat owners and the bond they have with their cats, it is important to understand the potential pathogenic diseases carried by the pet and how they can be transmitted to their owner (17). Domestic cats for example have the ability to transmit disease directly (i.e. petting, licking, physical injuries such as scratch or puncture) or indirectly (i.e. contamination of food or environment, inhalation, vector-borne, fecal-oral or soil-borne)(17,18). It is more likely that cats who carry disease are outdoor cats and younger cats (<1 year of age who have not yet developed their immunity)(17). Particularly susceptible to the transmission of disease from companion animal to human are the young (< 5 years of age), elderly, pregnant and/or immunocompromised (18).

Cats are also susceptible to tooth resorption, a condition known as feline oral resorptive lesions (FORL)(19). FORL are more prevalent than human resorptive lesions with a prevalence range of 28.5-67% (19). The etiology of FORL is also mostly idiopathic with some attributing the lesions to inflammation associated with periodontal disease or cementum deficiencies or possibly high levels of vitamin D uptake (19,20). Because radiographs are not routinely taken on cats, most FORL are diagnosed clinically and more often than not, the tooth cannot be saved and requires extraction (19).

Current research demonstrates the possibility of the transmission of oral bacteria from cats to owners (21). One study looked at the possible transmission of specific periodontal

pathogens and found the exact same species of *T. forsythia* in both cats and humans (21). It is possible that cats could transmit bacteria to their owners, but this is dependent on a number of factors including: survival of the microorganism in the environment, genetic factors of the microorganism, number of microorganisms that are shed, source and route of infection and frequency of contact (contact = cats licking face, eating from plates owner eats from, sharing food with cat, sleeping on the same pillow, etc)(21). Another study looking at the characterization of microbiota of healthy cats found that cats within the same household shared a similar microbiota which could indicate that animals and humans who cohabitate have the potential to share oral microbiota including those associated with infectious oral diseases (22). There is one study looking at the microbiota of cats with FORL (23), but no studies exist looking at the oral microbiome connection between humans with tooth resorption and cats.

One Health is the public health concept that human physical and mental health is interconnected with animal health and the health of our environment (24). One Health encourages all health and environmental professionals to work together to improve the lives of all species and one way in doing so is looking at cross-species disease transmission (24). Future steps in human health care, including oral health, need to look at the animals and the environment we all share.

By identifying the oral microbiota of humans with resorptive tooth lesions this pilot project will first identify the key human tooth resorptive-associated species. Second, the project will adopt a One Health approach and compare the oral microbiome of humans with resorptive lesions (obtained through sample collection) to the oral microbiome of felines (obtained through a database) to investigate a cross-species transmission of oral bacteria between felines and humans in one's environment. Thirdly the project will look at possible variables that may

influence the chances of cross-species transmission and alter the oral microbiota of humans with tooth resorption. The project has potential to encourage more research in this area.

Research Assumption

People with tooth resorption have a unique oral microbiome and may share bacterial species commonly found in cats. Increased contact with cats may increase the number of shared oral bacteria.

Research Objectives

1. To investigate the oral microbiome of humans with resorptive tooth lesions and compare it to the oral microbiome of humans without resorptive lesions.
2. To compare the oral bacterial species specific to humans with resorptive lesions with the oral microbiome of felines (from a database) to identify similar oral bacteria between the two species.
3. To investigate the variables which might influence or increase the opportunity for cross-species transmission of oral bacteria between humans and felines.

Chapter 2. Materials & Methods

2.1. Ethics

The research project underwent full Health Research Ethics Board (HREB) review from the University of Manitoba HREB board and received approval {HS22888 (H2019:220)}.

2.2. Study population

Adults with diagnosed resorptive tooth lesions confirmed by a dentist or dental specialist with radiographs were enrolled in the study from general practice dental clinics and specialty practices (endodontics, periodontics, orthodontics, prosthodontic & oral surgery). Participants were recruited through the above listed dental clinics. The dental clinics were sent information about the study through email, social media, presentations and by word of mouth about what participation in the study would involve. Potential participants were informed about the study from the dental provider and if interested in participating in the study, the dental provider asked if the investigator could contact the patient. The investigator provided the participant with all study information and invited the patient to participate. Consent was obtained from each participant. Adults without resorptive lesions but matched for age and gender (control group) were enrolled in the study in the same manner as explained above.

Inclusion Criteria: Adults 18 years of age and older, current or history of oral resorptive lesion (external or internal), single or multiple resorptive lesions, no resorptive lesions (control).

Exclusion Criteria: Use of antibiotics in previous 6 months, pregnant, current tobacco user, current orthodontic treatment, current endodontic treatment.

A total of 20 samples were collected: 10 case samples and 10 control samples.

2.3 Sample collection

Using an interdental brush, plaque was collected from all available supra gingival tooth surfaces. To disperse the plaque from the interdental brush, the brush was submerged into a collection tube with Qiagen RNA Protect reagent and then twirled for 5 seconds before discarding the interdental brush. The procedure was repeated with a new interdental brush and the plaque was dispersed in the same tube. Samples were stored at -80°C until ready to be processed. Samples were then sent to Microbiome Insights for DNA extraction, PCR, sequencing and sequence processing.

2.4 Microbial analyses

2.4.1.DNA extraction, PCR, sequencing, and sequence processing

Deep sequencing of the small ribosomal rRNA subunit (16S rRNA) gene was utilized to survey the bacterial community and gain insights on the population dynamics. This was achieved by taking thousands of copy variants of the 16S rRNA gene and clustering the sequence data into count data in the form of Operational Taxonomic Units (OTUs).

Specimens were placed into a MoBio PowerMag Soil DNA Isolation Bead Plate. DNA was extracted following MoBio's instructions on a KingFisher robot. Bacterial 16S rRNA genes were PCR-amplified with dual-barcoded primers targeting the V4 region (515F 5'-GTGCCAGCMGCCGCGGTAA-3', and 806R 5'-GGACTACHVGGGTWTCTAAT-3')(25). Amplicons were sequenced with an Illumina MiSeq using the 300-bp paired-end kit (v.3). Sequences were denoised, taxonomically classified using Silva (v. 138) as the reference database, and clustered into 97%-similarity operational taxonomic units (OTUs) with the mothur software package (v. 1.44.1)(26), following the recommended procedure (27).

2.4.2 Quality Control

The potential for contamination was addressed by co-sequencing DNA amplified from specimens and from template-free controls (negative control) and extraction kit reagents processed the same way as the specimens. A positive control from 'S00Z1-' samples consisting of cloned SUP05 DNA, was also included. Operational taxonomic units were considered putative contaminants (and were removed) if their mean abundance in controls reached or exceeded 25% of their mean abundance in specimens.

2.4.3 Statistical analysis of oral microbiome results

Alpha diversity was estimated with the Shannon index on raw OTU abundance tables after filtering out contaminants. The significance of diversity differences was tested with ANOVA or linear mixed model depending on the study design.

Beta diversity is a measure of how similar or dissimilar the samples are and is usually represented by a distance matrix which is then used to do Principal Coordinates Analysis (PCoA)(28). The result of this is an ordination plot of multiple dimensions, where each sample is a point and the distance between the points represents the similarity of those samples (closer together = more similar)(28). Differences in microbial abundances between two samples (e.g., at species level) values are from 0 to 1; 0 means both samples share the same species at exact the same abundances & 1 means both samples have completely different species abundances.(29) To estimate beta diversity across samples, OTUs occurring with a count of less than 3 in at least 10 % of the samples were excluded and then Bray-Curtis indices were computed. Beta diversity, emphasizing differences across samples, was visualized using Principal Coordinate Analysis (PCoA) ordination. Variation in community structure was assessed with permutational multivariate analyses of variance (PERMANOVA) with the case group as the main fixed factor

and using 9999 permutations for significance testing. All analyses were conducted in the R environment.

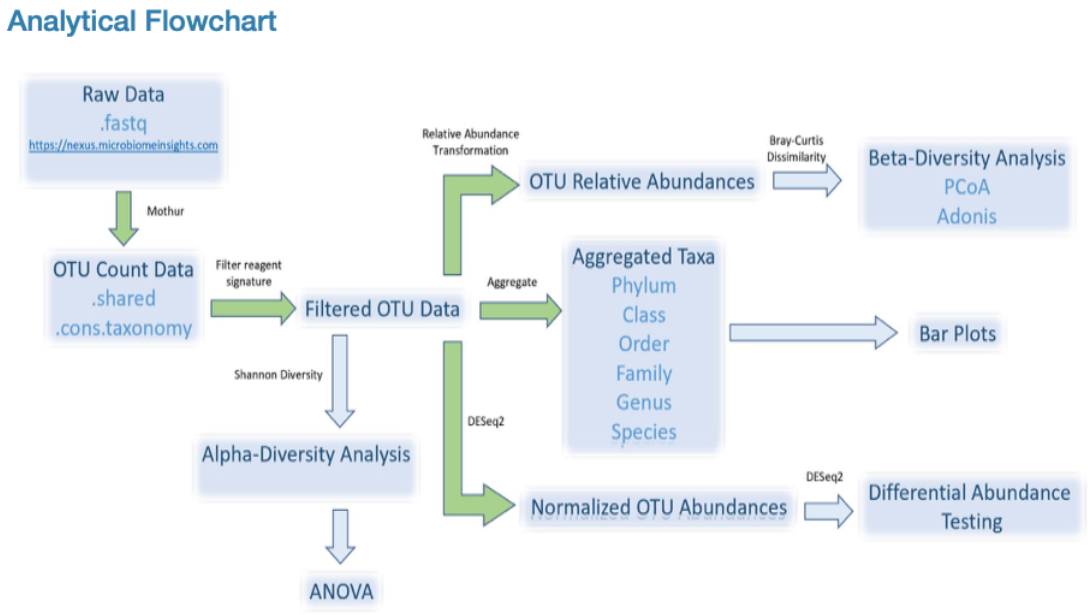

Figure 3: Analytical flowchart of statistical interpretations of data.

2.5 Statistical analysis comparing human oral microbiome to feline oral microbiome

Objective 2 involved a descriptive comparison of the list of human oral bacterial species identified as significant in the analysis for Objective 1 to the reported presence of these species in the oral microbiome of felines (from a study database). The species of significance was narrowed down to a smaller group of interest, only some of which would be common between people with tooth resorptive lesions and cats.

2.6 Methodology for descriptive data

2.6.1 Questionnaire

At the time of sample collection, participants with and without resorptive lesions completed a questionnaire. The questions are to assist in identifying any human factors influencing a change in tooth resorptive oral microbiota and the factors that may influence the oral microbiota of felines and cross-species transmission. Questions were a combination of dichotomous, multiple choice (single answer and multiple answers), Likert scale and open ended. See Appendix for copy of questionnaire.

2.6.2 Statistical analysis of descriptive data

For objective 3, Chi-square, Fisher Exact Test and T-test analyses were attempted to identify clinical or environmental variables which might be modify the observed association between the presence or absence tooth resorption between the experimental and control groups. Data analysis was performed using NCSS 2020 Statistical Software (Kaysville, Utah) and Vasserstats (R.Lowry, VassarStats: Website for Statistical Computation, http://vassarstats.net). In general, p-values less than 0.05 were considered as significant, but without correction for multiple inference in this exploratory pilot study. Any findings will be considered reported as hypothesis generating rather than confirmatory.

Chapter 3. Results

The first step in the study was to identify humans with tooth resorption. This required collaboration with dentists, dental specialists and dental hygienists who were able to assist in identifying 10 humans with tooth resorption from Winnipeg and surrounding areas (see Table 1). 10 matching controls for age and sex and no history of tooth resorption were identified in the same manner as described above and included in the study.

Table 1: Age, sex, and tooth resorption demographics of participants.

PARTICIPANTS	AGE (YEAR)	GENDER	TYPE OF RESORPTIVE TOOTH LESION
EXPERIMENTAL GROUP			
1	> 65	F	External
2	45–54	M	External
3	> 65	M	Internal
4	55–64	F	External
5	45–54	F	Internal
6	45–54	M	Internal
7	55–64	M	External
8	25–34	M	External
9	18–24	M	Internal
10	45–54	F	External
CONTROLS			
11	> 65	F	N/A
12	> 65	M	N/A
13	18–24	M	N/A
14	45–54	M	N/A
15	25–34	M	N/A
16	55–64	F	N/A
17	55–64	M	N/A
18	45–54	F	N/A
19	45–54	M	N/A
20	45–54	F	N/A

Plaque samples were collected from each participant and participants completed a questionnaire. Plaque sample analysis and participant questionnaire analysis were completed to fulfill all three study objectives.

3.1 Investigation of the oral microbiome of humans with and without tooth resorption

Each experimental group and control group oral sample was frozen at -80°C in RNA Protect immediately after collection until all 20 oral samples (10 experimental and 10 control) were collected and ready to be sent to Microbiome Insights Laboratory for further analysis.

3.1.1 Sequence Curation & Metrics

Microbiome Insights performed deep sequencing of the 16S rRNA genes of bacteria within the oral microbiome of all samples. To perform sequencing, amplicons were first generated. Reverse-transcription-PCR was performed on the oral microbiome samples. The resulting PCR fragments, termed amplicons, were then sequenced. During data analysis, the resulting sequencing reads were aligned to each other and those sufficiently similar in sequence above a certain threshold (97% sequence similarity was used for the samples) were grouped together and counted. These groups are termed operational taxonomic units (OTUs). When using the 97% threshold, each OTU is loosely representative of a bacterial species (note that a lower similarity threshold would be used for an OTU representing a bacterial genus). These OTUs were then compared between the two oral microbiomes.

The resulting dataset had 3352 OTUs (including those occurring once with a count of 1). An average of 10971 quality-filtered reads were generated per sample. For the control group, the minimum to maximum read count ranged from 1,000 reads to one with 18,000 reads with a median read count of ~8,000, while for the experimental group, the read count ranged from

1,000 reads to one with 25,000 reads with a median read count of ~10,000. Sequencing quality for R1 and R2 was determined using FastQC 0.11.5, and visualized below in Figure 4.

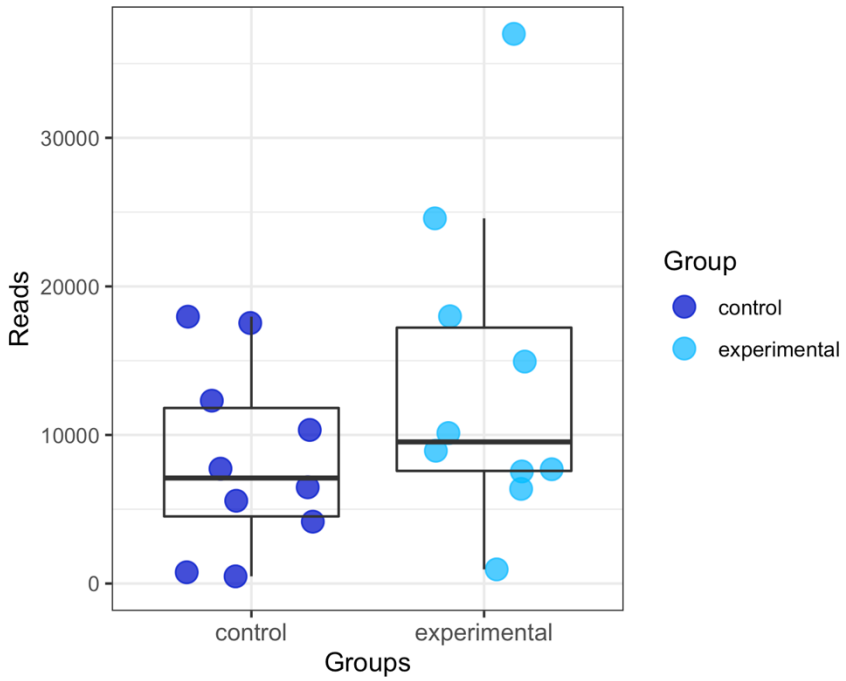

Figure 4: The following box-and-whisker plot illustrates the total number of quality filtered reads per sample. These reads reflect the total number of high-quality sequences that align with 16Sv4, clustered into OTUs and were assigned taxonomic classification. Any ambiguous or low-quality data were discarded from the subsequent analyses.

Three samples were dropped from the analysis due to low read counts; 1 from the experimental group and 2 from the case group reducing the total sample size from 20 to 17 (see Table 2).

Table 2: Summary of the total number of quality filtered reads per sample. Red indicates the samples which were dropped from the analysis.

GROUP	READS
Experimental	24580
Experimental	10127
Experimental	**950**
Experimental	36999
Experimental	7691
Experimental	8936
Experimental	7544
Experimental	6367
Experimental	17986
Experimental	14940
Control	4163
Control	17962
Control	**482**
Control	6475
Control	7731
Control	5569
Control	12308
Control	**757**
Control	10336
Control	17535

3.1.2 Taxonomic Composition

To provide each OTU with taxonomic classification, the high-quality sequencing reads were aligned to reference sequences in the Silva v. 138 database. The function of this database is specifically to provide reference sequences for rRNA genes from known source species. Figure 5 looks at the four most abundant phyla identified across both sets of samples (control and experimental). These were Actinobacteriota, Bacteroidota, Firmicutes, and Proteobacteria. Within each of these phyla, the relative abundance of the top 5 most abundant genera in both sets of samples (control and experimental) were plotted.

Based on this graph, there were no statistically significant differences between the mean proportions of OTUs assigned to any of the genera between the control and experimental group.

Visually, this is seen by the overlapping error bars and the fact that there are no markers of significance indicated on the graph.

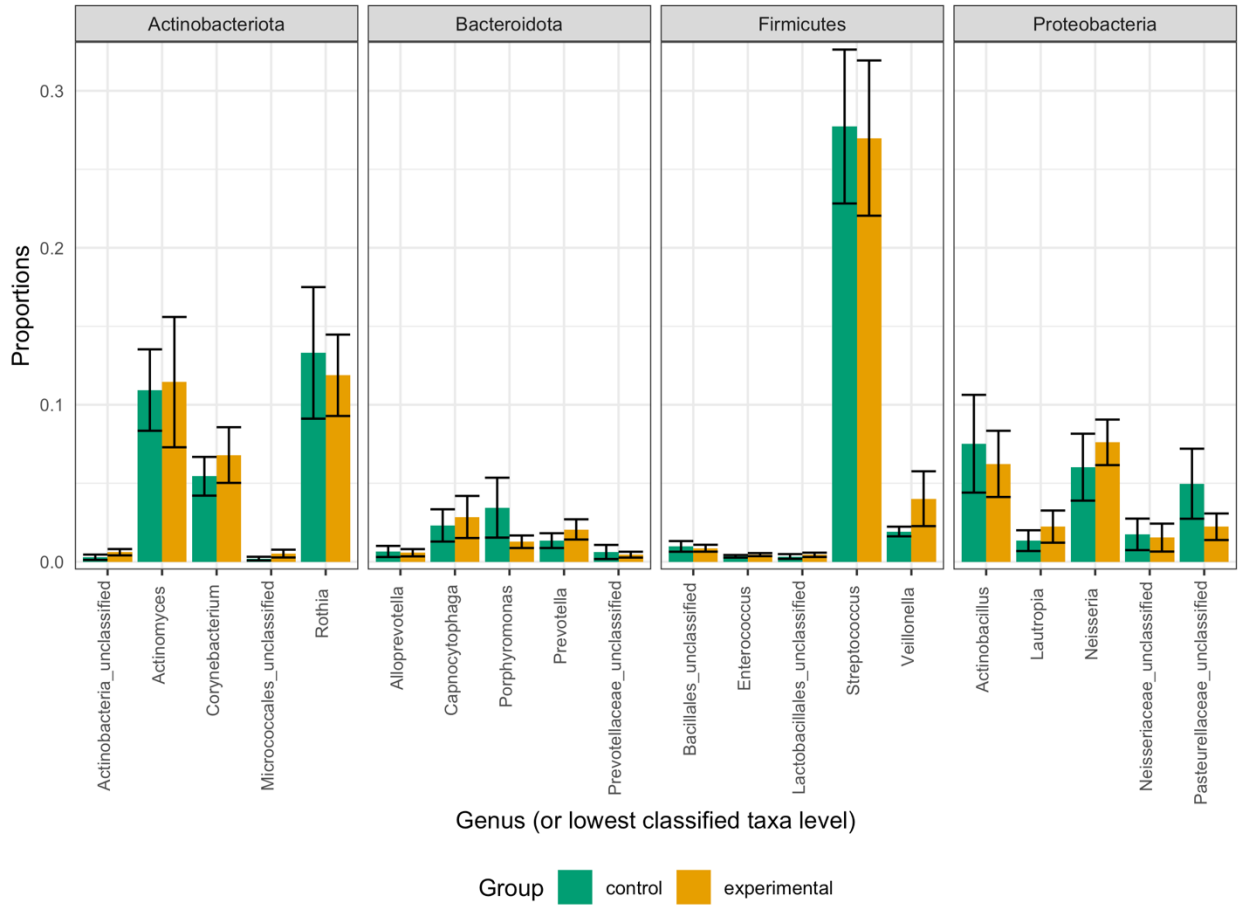

Figure 5: Illustration of the mean and standard error of the relative abundances of the 5 most abundant genus-level taxa within the 4 most abundant Phyla. The Genus-level plots are grouped according to Phylum along the x-axis. The groupings along the y-axis represent the column of metadata. The barplot colors represent the 2 groups: experimental and control.

Alternatively, the taxa were aggreagted at each taxonomic rank (Figure 6; Phylum, Figure 7; Class, Figure 8; Order, Figure 9; Family, Figure 10; Genus) to further see the different proportions of taxa. These “stacked bar plot” graphs with the y-axis goin from 0% to 100% are less informative, but provide a visual for the data. For each of the 17 samples, the individual graphs illustrate the relative proportions of the OTUs assigned to each of the 10 most abundant

phyla found across all of the samples. As seen in each figure, there is quite a lot of variation between samples in the same group (experimental or control), and similar levels of variation are apparent between these two groups.

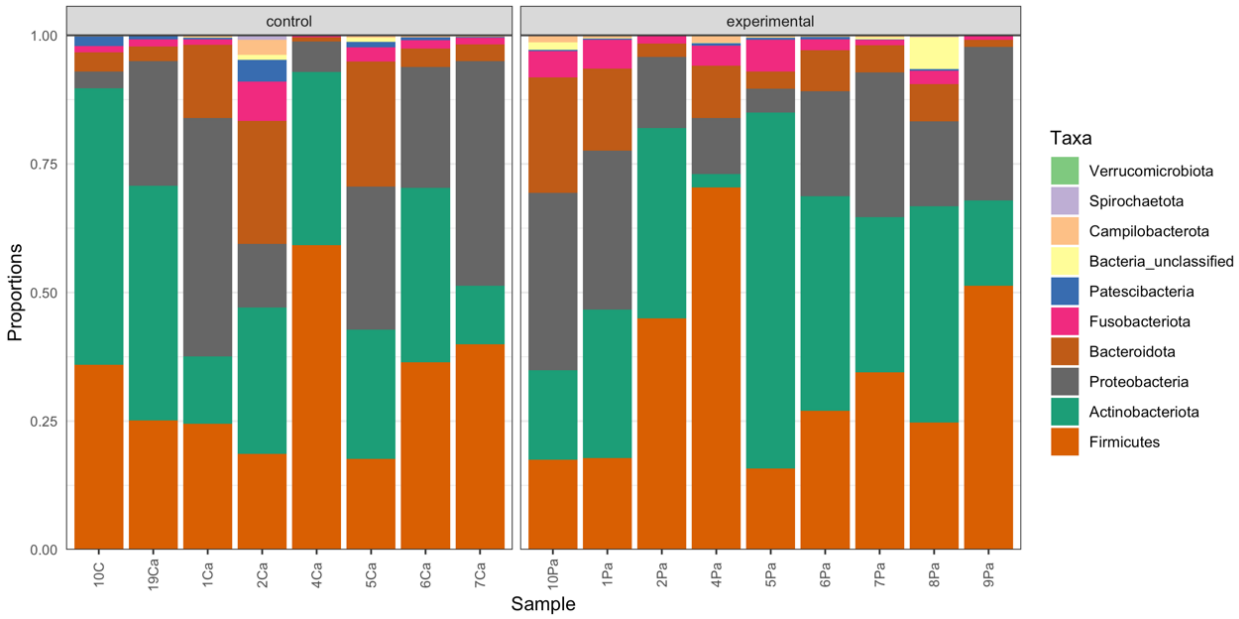

Figure 6: Per-sample taxonomic composition of Phylum.

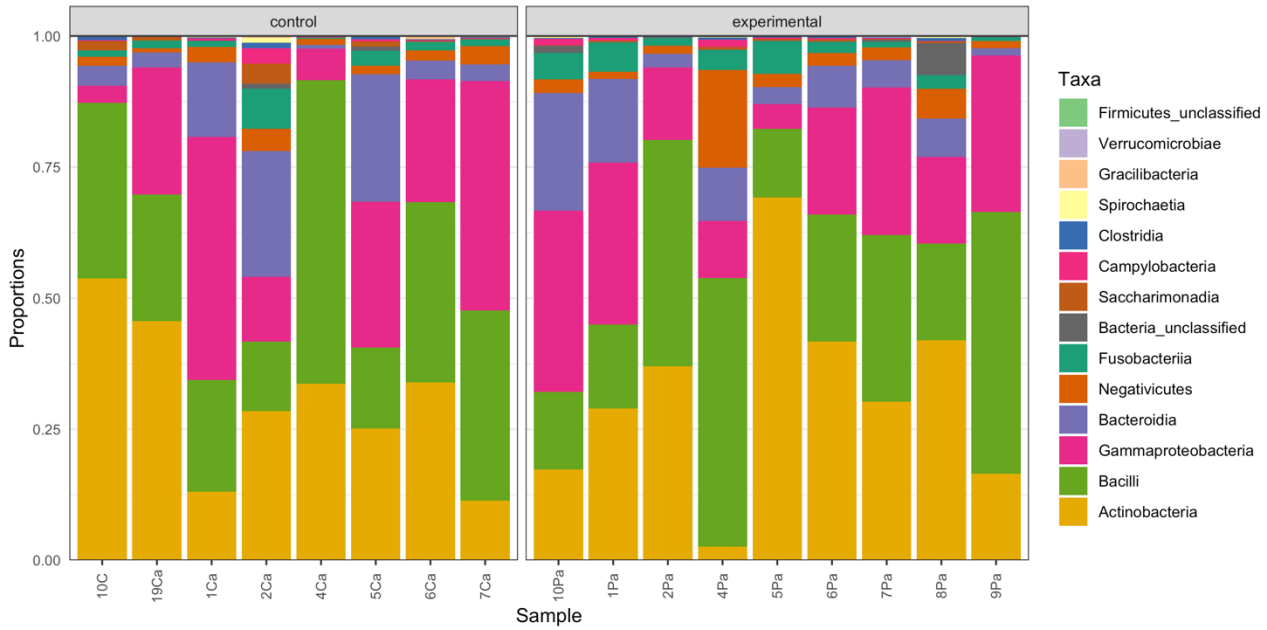

Figure 7: Per-sample taxonomic composition of Class.

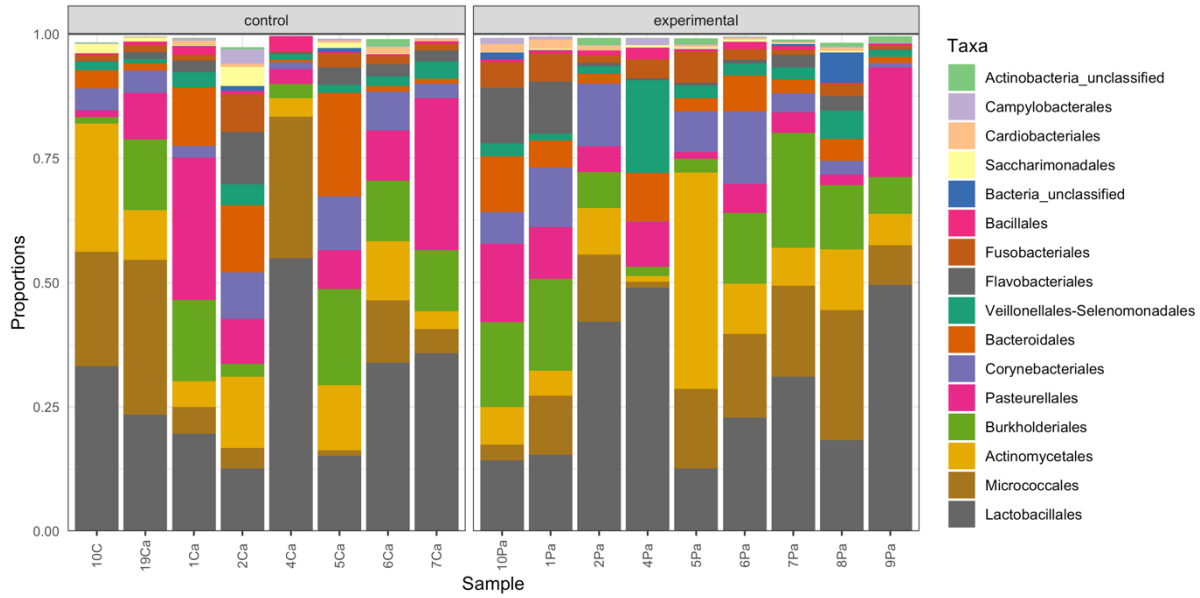

Figure 8: Per-sample composition of Order. The unfilled portion of the bar-plots represent lower-abundance taxa.

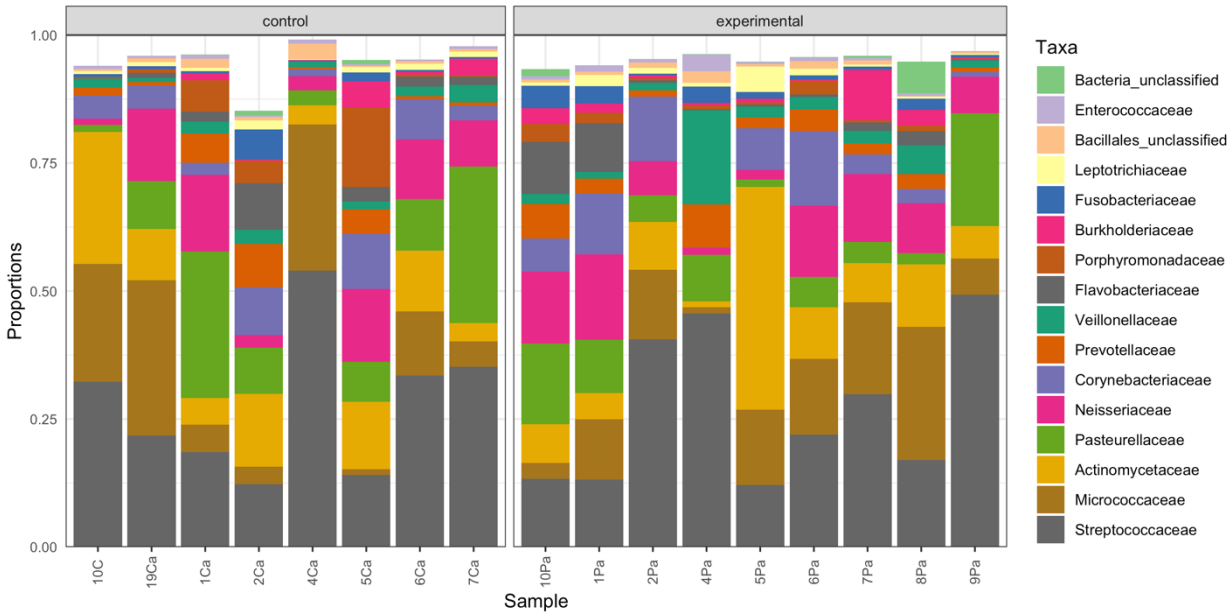

Figure 9: Per-sample taxonomic composition of Family. The unfilled portion of the bar-plots represent lower-abundance taxa.

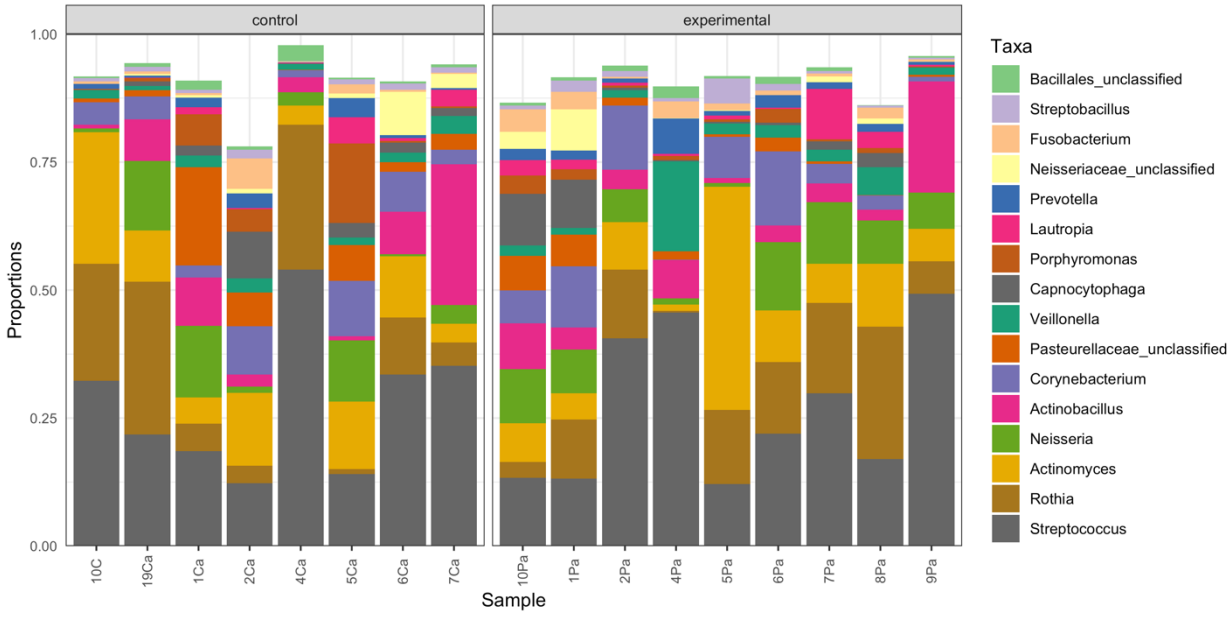

Figure 10: Per-sample taxonomic composition of Genus. The unfilled portion of the bar-plots represent lower-abundance taxa.

Looking at taxonomic abundancies at the genus level, the average proportions for each genus taxon were identified across the 9 experimental samples and 8 control samples and the mean, standard deviation, and coefficient of variation (CV; a percentage calculated as Standard Deviation/Average * 100) were calculated for each. The standard deviation and CV values demonstrated how scattered the data are, and for both groups, these values are very high, with the CV > 50% for almost all taxa and many that had a CV > 100% (Table 3). This means that within each group, the data were so scattered that no statistically significant difference between the means of the groups were found. The six most abundant genera identified in both the experimental and control groups were *Streptococcus, Rothia, Actinomyces, Actinobacillus, Neisseria* and *Corynebacterium*.

Table 3: Mean Genera abundancies standard deviation and coefficient of variance for experimental and control groups.

Genus name	Experimental			Control		
	Average abundance	Standard deviation	Coefficient of variance	Average abundance	Standard deviation	Coefficient of variance
Gammaproteobacteria_unclassified	0.00285	0.00289	101.22814	0.00280	0.00184	65.73943
Streptococcus	0.26988	0.14837	54.97516	0.27725	0.13866	50.01315
Rothia	0.11876	0.07775	65.47305	0.13306	0.11855	89.09914
Actinomyces	0.11442	0.12450	108.80901	0.10936	0.07337	67.08905
Actinobacillus	0.06232	0.06331	101.57992	0.07516	0.08809	117.20677
Neisseria	0.07605	0.04352	57.22066	0.06022	0.06035	100.20410
Corynebacterium	0.06795	0.05318	78.26190	0.05443	0.03485	64.03174
Veillonella	0.04014	0.05243	130.60556	0.01923	0.00870	45.24345
Pasteurellaceae_unclassified	0.02227	0.02535	113.81231	0.04968	0.06310	127.00381
Fusobacterium	0.01862	0.01500	80.52282	0.01243	0.01940	156.03609
Porphyromonas	0.01271	0.01198	94.26772	0.03443	0.05398	156.75683
Prevotella	0.02057	0.01929	93.75423	0.01344	0.01336	99.39191
Neisseriaceae_unclassified	0.01536	0.02670	173.77058	0.01743	0.02840	162.98085
Capnocytophaga	0.02850	0.04026	141.29253	0.02312	0.02913	125.99299
Lautropia	0.02236	0.03073	137.43762	0.01341	0.01867	139.25587
Bacillales_unclassified	0.00860	0.00662	77.05740	0.00976	0.00961	98.49828
Cardiobacterium	0.00667	0.00679	101.75195	0.00475	0.00443	93.28387

Saccharimonadales_ge	0.00248	0.00186	74.89749	0.00964	0.01328	137.70975
Enterococcaceae_unclassified	0.00465	0.00752	161.77971	0.00163	0.00209	128.46511
Enterococcus	0.00453	0.00289	63.79776	0.00346	0.00236	68.10985
Alysiella	0.00282	0.00341	121.08788	0.01035	0.01075	103.81549
Alloprevotella	0.00572	0.00691	120.86410	0.00652	0.00999	153.15118
Lactobacillales_unclassified	0.00439	0.00410	93.40802	0.00334	0.00425	127.12865
Micrococcaceae_unclassified	0.00382	0.00314	82.14950	0.00344	0.00427	123.82642
Campylobacter	0.00441	0.00513	116.17482	0.00543	0.00963	177.26732
Bacteria_unclassified	0.00897	0.02015	224.58499	0.00235	0.00408	173.42449
Weeksellaceae_unclassified	0.00454	0.00441	97.10573	0.00551	0.00388	70.40622
Prevotellaceae_ge	0.00434	0.00357	82.26398	0.00253	0.00389	153.44480
Streptobacillus	0.01330	0.01468	110.40297	0.00912	0.00482	52.80976
Actinobacteria_unclassified	0.00605	0.00601	99.39521	0.00283	0.00482	170.49002
Micrococcales_unclassified	0.00518	0.00746	143.97241	0.00197	0.00332	168.44017
Prevotellaceae_unclassified	0.00449	0.00556	123.75405	0.00620	0.01273	205.31578
Veillonellaceae_unclassified	0.00126	0.00259	205.21730	0.00030	0.00035	115.17484
Selenomonas	0.00153	0.00204	133.50651	0.00293	0.00529	180.61075
Bacteroides	0.00144	0.00130	90.46178	0.00263	0.00603	229.47397
Tannerella	0.00085	0.00128	149.38480	0.00134	0.00221	165.15515
Bacteroidales_unclassified	0.00153	0.00228	148.45428	0.00018	0.00051	282.84271
Absconditabacteriales_(SR1)_ge	0.00037	0.00076	208.62834	0.00155	0.00190	122.58891
Treponema	0.00080	0.00092	114.59399	0.00121	0.00303	251.39019
Lachnoanaerobaculum	0.00048	0.00043	90.72131	0.00142	0.00192	135.61368
Verrucomicrobiae_unclassified	0.00050	0.00049	96.98518	0.00046	0.00055	118.93064
Pseudopropionibacterium	0.00070	0.00117	165.94957	0.00067	0.00103	154.24106
Oribacterium	0.00079	0.00136	170.89187	0.00001	0.00003	282.84271
Clostridia_UCG-014_ge	0.00022	0.00037	168.81499	0.00057	0.00087	153.93094
Bifidobacterium	0.00035	0.00036	104.47575	0.00045	0.00122	268.45676
Faecalibacterium	0.00043	0.00041	94.39327	0.00067	0.00052	76.81250
Blautia	0.00018	0.00019	104.94828	0.00041	0.00071	172.09794
Firmicutes_unclassified	0.00034	0.00069	202.82942	0.00020	0.00026	131.69773
Lachnospiraceae_unclassified	0.00015	0.00024	163.01437	0.00049	0.00070	141.25464
Parvimonas	0.00020	0.00037	188.26418	0.00013	0.00022	164.55779
Enterobacteriaceae_unclassified	0.00013	0.00023	170.01863	0.00005	0.00012	227.81169

3.1.3 Alpha Diversity

Alpha diversity is a measure of richness (how many OTUs) and evenness (how evenly distributed these OTUS are) in a sample (28). The alpha diversity, also called the Shannon diversity index, is a mathematical way to express the diversity (or balance) of species ***within*** a microbiome, community, or sample. It does not only take into account the total number of different species present, it also takes into account their relative abundance (28). The Shannon index has been calculated for each of the samples in the control and experimental groups based on the number of different species identified in the sample (where each OTU cluster with >97% similarity represents a species) and the relative abundance of these species in each group (the number of OTUs assigned to each species), and the mean Shannon index was then compared between the experimental and control groups to see whether they exhibited different levels of species diversity. Values for the Shannon diversity index can range from 1 (in the case of a single dominant species) up to the total number of all species present in the sample (in the case of all species having equal abundance)(30).

Figure 11 illustrates the alpha diversity (Shannon index) for each sample. The summary statistics tabulated (Table 4), demonstrates the control and experimental groups have similar mean Shannon index values (2.693 and 2.791, respectively), and ANOVA analysis (Table 5) shows that there were no statistically significant differences between the Shannon index values of the two groups ($P > 0.05$).

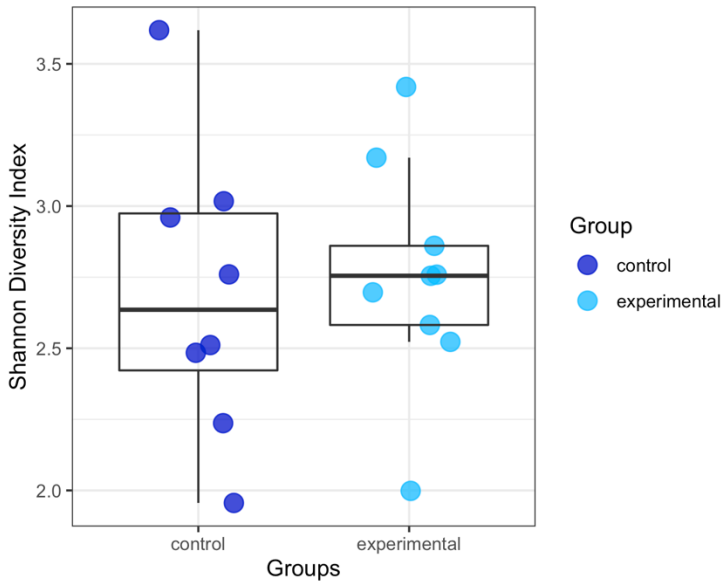

Figure 11: Shannon index showing measure of richness and evenness in each sample. The graph in this section plots the Shannon index on the y-axis for each sample vs. the number of OTUs assigned to each species in that sample.

Table 4: Shannon diversity summary statistics.

GROUP	SHANNON MEAN	SHANNON SD
CONTROL	2.693	0.516
EXPERIMENTAL	2.751	0.401

Table 5: ANOVA results for Shannon diversity.

	DF	F VALUE	PR(>F)
GROUP	1	0.069	0.796
RESIDUALS	15	NA	NA

These results indicate there is the same level of diversity in each set of samples with no significant difference. Therefore, the experimental and control groups showed similar microbiome diversity at the genus level.

3.1.4 Beta Diversity

Beta diversity is a mathematical method for comparing the diversity (or balance) of species between microbiomes, communities, or samples (28). The values for beta diversity range from 0 to 1: a value of 0 indicates that the two groups share the same species at the same abundances, while a value of 1 indicates that the groups contain completely different species (29). There are different ways to calculate beta diversity. In this study, the Bray–Curtis dissimilarity method was applied. This method also considers both the total number of different species present and their relative abundance. All profiles were inter-compared in a pair-wise fashion to determine a dissimilarity score and store it in a distance dissimilarity matrix. Distance functions produced low dissimilarity scores when comparing similar samples. Abundance-weighted sample pair-wise differences were calculated using the Bray-Curtis dissimilarity. Bray-Curtis dissimilarity is calculated by the ratio of the summed absolute differences in counts to the sum of abundances in the two samples (31).

To obtain a graphical representation of microbiome composition similarity among samples, OTU abundances were summarized into Bray-Curtis dissimilarities and a Principal Coordinate Analysis ordination (PCoA) was performed. PCoA provides a way to visualize similarities or dissimilarities between samples (29). With the Bray–Curtis method, we are looking at dissimilarities. This conclusion may be correct since this measure is one of species diversity.

To do a PCoA, a matrix was first generated of the differences (or "distances") between each of the pairwise comparisons for each of the 17 samples. This matrix is called the Bray–Curtis dissimilarity matrix. PCoA then mathematically transforms the dissimilarity matrix into a smaller number of variables called the principal coordinates. The process of mathematical transformation assigned each of the values in the matrix to a position in low-dimensional space. Figure 12 shows the data reduced to two dimensions as there are two principal coordinates, PCoA 1 and PCoA2. The samples in neither the experimental group nor the control group showed any clustering on the PCoA graph. Instead, the samples are scattered in position across the graph. Therefore, just looking at the PCoA graph, it can be inferred that the beta diversity among the two groups was similar.

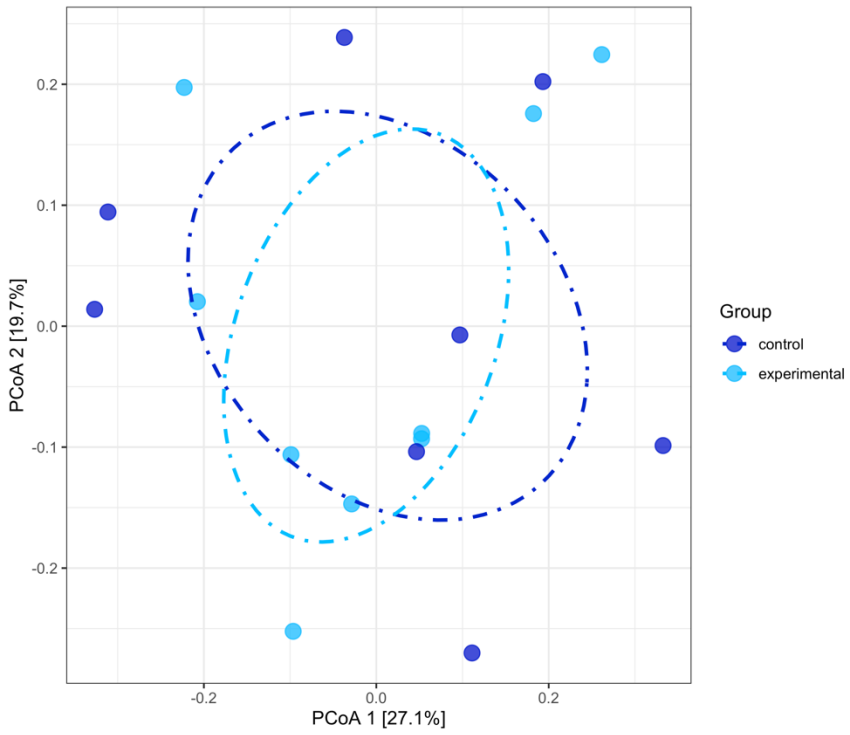

Figure 12: Ordination of microbiome composition for all specimens according to Bray-Curtis dissimilarities.

The PERMANOVA (permutational analysis of variance) results showed that this was the case ($P > 0.05$). PERMANOVA is a type of ANOVA. Whereas normal ANOVA is used to check for statistically significant differences among the sample means (average value) between different groups, PERMANOVA looks at whether there is a significant difference in the positioning of the sample data points in space between groups, based on the centroid (mean position in 2D space) and dispersion (degree to which the data points are scattered) for each group.(32) Note that PERMANOVA does not use the outcome of the PCoA analysis; instead, it uses the original distance matrix as the input.

The two ellipses drawn on the graph are confidence ellipses and there is one for each group. The ellipses represent the space on the PCoA plot in which we have 95% confidence/certainty that the true mean (or centroid in this case) lies within. The ellipses in Figure 12 are broad on the graph, which reflects the high dispersion of the data points across the PCoA plot.

Therefore, the PCoA analysis visually indicates that the experimental and control groups do not have a dissimilar beta diversity while the PERMANOVA analysis mathematically demonstrated that there is indeed no statistically significant difference between the beta diversity of the two groups ($P > 0.05$) in Table 6.

Table 6: PERMOANOVA analysis of beta diversity.

	F VALUE	**PR(>F)**
GROUP	0.0302	0.9377
RESIDUALS	0.9698	NA
TOTAL	1.0000	NA

3.1.5 Differential Abundance Testing

Differential abundance testing was used to detect differences in species abundance (based on the OTU counts) between the experimental and control groups. There are three main steps in this type of analysis: taxonomic classification (performed when the OTUs are generated), counting the number of OTUs identified per taxonomic unit, and performing statistical analyses on these data to determine whether there are any significant differences between the groups.

For the statistical analysis step in differential abundance testing, it was performed with the R package, DESeq2, using negative binomial distribution and the likelihood-ratio test. The result is that there are no OTUs that have a significantly different abundance between the experimental and control groups. In other words, there are no significant differences in the species abundance between the two groups.

3.2 Comparison of oral microbiome of humans with resorptive lesions to oral microbiome of felines

To investigate the possibility of similar oral bacteria between humans with tooth resorption and felines, the oral microbiome results from objective 1 were compared with the oral microbiome of felines from two studies which characterized the oral bacteria of felines (23,33).

3.2.1 Feline oral microbiome

A 2015 study by Dewhirst et al. identified the feline oral bacterial species present and generated a list of reference sequences for future next generation sequencing studies (33). In this study by Dewhirst et al., they identified 246 full-length 16S rRNA sequences representing a total of 171 different bacterial taxa, but not the abundancies of each species (33). These oral bacteria were isolated from 20 cats aged between one and seven years old. Ten of the cats were periodontally healthy and ten had periodontitis (33). Samples taken from different sub gingival

areas of the mouth cavity were pooled for individual cats, and the samples from the two groups were then pooled in turn (33). This resulted in two DNA pools, one from the ten periodontally healthy cats and one from the ten periodontally diseased cats. Each DNA pool was amplified to make clone libraries using primers toward 16S rRNA (33).

Feline oral taxa in 11 phyla were identified in this study: Firmicutes, 72; Proteobacteria, 38; Bacteroidetes, 26; Spirochaetes, 16; Actinobacteria, 10; Synergistetes, 4; Chlorobi, 1; Chloroflexi, 1; Fusobacteria, 1; SR1, 1; and TM7, 1 (33). The results from the Dewhirst et al study suggest that the "feline oral microbiota are largely conserved between cats at the phylum level, and that the population is highly diverse, rich and even. A strong core microbiome was evident among all cats, yet significant differences in oral bacterial populations were observed across cats in each household." (33)

A 2021 study by Thomas et al is the first of its kind to compare the feline oral microbiome of healthy cats to the microbiome of cats with FORL (23). Supra gingival oral plaque samples were taken from 25 healthy cats and 40 cats with FORL (23). DNA was extracted, the V4 region of the 16S rRNA gene was amplified by PCR and the amplicons were sequenced (23). The study identified six genera unique to the oral microbiome of healthy cats and 18 genera unique to the oral microbiome of cats with FORL (Table 7)(23). The study found no association between any specific bacterial species and FORL and further to that there were no oral microbiome species differences between orally healthy cats and cats with FORL (23). No abundancies or complete data sets were available from the study.

Table 7: Feline oral microbiome genera unique of cats who are orally healthy and who have FORL (23).

ORAL HEALTH	FORL
Bergeyella	*Acetitomaculum*
Fusibacter	*Actinomyces*
Gammaproteobacteria	*Alysiella*
Methylobacterium	*Bacteroides*
Moraxella	*Bergeyella*
Porphyromonas	*Catonella*
	Clostridium
	Fusibacter
	Helicobacter
	Leptotrichia
	Methanimicrococcus
	Moraxella
	Peptococcus
	Prevotella
	Rothia
	Sphingomonas
	Staphylococcus
	Treponema

3.2.2 Comparison of feline and human oral microbiome

For the reason described in 3.1 that there were no significant differences in the species of bacteria comprising the oral microbiome of people with and without resorptive tooth lesions, a comparison was made between all human oral samples collected and the feline oral microbiome data from the Dewhirst et al study and from the Thomas et al study.

The cat microbiome data from the Dewhirst study was first ordered into phylum groups. The sequences of the FOT clones/strains mostly aligned to reference sequences from the phyla Firmicutes, Proteobacteria, Bacteroidota, Spirochaetota, Actinobacteriota, and Fusobacteriota. These are five of the same top six phyla identified in the human oral microbiome data across both the experimental and control groups as per the results in objective 1: Actinobacteriota, Bacteroidota, Firmicutes, Fusobacteriota, and Proteobacteria (Table 8).

Table 8: Top phyla for oral microbiome and feline oral microbiome

Top 6 human oral phyla	**Top 6 feline oral phyla**
1. Actinobacteriota	1. Firmicutes
2. Bacteroidota	2. Proteobacteria
3. Firmicutes	3. Bacteroidota
4. Proteobacteria	4. Spirochaetota
5. Fusobacteriota	5. Actinobacteriota
6. Patescibacteria	6. Fusobacteriota

Comparing all genera between the human oral microbiome samples and feline oral microbiome data from the Dewhirst et al and Thomas et al studies, there are 18 common genera between humans and felines (highlighted in yellow), *Bergeyella, Catonella, Moraxella* and *Peptococcus* are four common to cat genera only (highlighted in green) and *Alysiella, Prevotella* and *Rothia* are three genera common in the human oral microbiome study results and the microbiome of cats with FORL (highlighted in blue) (Table 9).

Table 9: Common genera between human oral microbiome and all feline oral microbiome (yellow), common genera between cats with and without FORL (green) & common oral genera between humans and cats with FORL (blue) (23,33)

Human genera identified (current study)	**Cat genera identified** (33)	**Cat FORL genera identified** (23)
Absconditabacteriales_(sr1)_ge	*Acholeplasmatales*	*Acetitomaculum*
Actinobacillus	*Actinobacteria*	*Actinomyces*
Actinobacteria_unclassified	*Actinomyces*	*Alysiella*
Actinomyces	*Alloprevotella*	*Bacteroides*
Alloprevotella	*Anaerolineae*	*Bergeyella*
Alysiella	*Aquaspirillum*	*Catonella*
Bacillales_unclassified	*Bacteroides*	*Clostridium*
Bacteria_unclassified	*Bacteroidia [G-2]*	*Fusibacter*
Bacteroidales_unclassified	*Bacteroidia [G-4]*	*Helicobacter*
Bacteroides	*Bacteroidia [G-5]*	*Leptotrichia*
Bifidobacterium	*Bacteroidia [G-6]*	*Methanimicrococcus*
Blautia	*Bergeyella*	*Moraxella*
Campylobacter	*Brachymonas*	*Peptococcus*
Capnocytophaga	*Campylobacter*	*Prevotella*
Cardiobacterium	*Capnocytophaga*	*Rothia*
Clostridia_ucg-014_ge	*Cardiobacterium*	*Sphingomonas*
Corynebacterium	*Catonella*	*Staphylococcus*
Enterobacteriaceae_unclassified	*Chlorobi*	*Treponema*
Enterococcaceae_unclassified	*Clostridiales [F-1][G-1]*	
Enterococcus	*Clostridiales [F-1][G-2]*	
Faecalibacterium	*Clostridiales [F-2][G-1]*	
Firmicutes_unclassified	*Clostridiales [F-3][G-1]*	
Fusobacterium	*Clostridiales III [G-3]*	
Gammaproteobacteria_unclassified	*Clostridiales IV [G-1]*	
Lachnoanaerobaculum	*Comamonas*	
Lachnospiraceae_unclassified	*Conchiformibius*	
Lactobacillales_unclassified	*Corynebacterium*	
Lautropia	*Desulfobulbus*	
Micrococcaceae_unclassified	*Desulfomicrobium*	
Micrococcales_unclassified	*Desulfovibrio*	
Neisseria	*Desulfovibrionales [G-1]*	
Neisseriaceae_unclassified	*Enterococcus*	
Oribacterium	*Erysipelotrichaceae [G-1]*	
Parvimonas	*Filifactor*	
Pasteurellaceae_unclassified	*Finegoldia*	
Porphyromonas	*Fretibacterium*	
Prevotella	*Fusobacterium*	
Prevotellaceae_ge	*Globicatella*	
Prevotellaceae_unclassified	*Helcococcus*	
Pseudopropionibacterium	*Lachnospiraceae XIVa [G-2]*	
Rothia	*Lachnospiraceae XIVa [G-3]*	
Saccharimonadales_ge	*Lachnospiraceae XIVa [G-5]*	

Selenomonas	*Lachnospiraceae XIVa [G-6]*	
Streptobacillus	*Leptospiraceae*	
Streptococcus	*Leucobacter*	
Tannerella	*Luteimonas*	
Treponema	*Moraxella*	
Veillonella	*Neisseria*	
Veillonellaceae_unclassified	*Ottowia*	
Verrucomicrobiae_unclassified	*Parvimonas*	
Weeksellaceae_unclassified	*Pasteurella*	
	Pasteurellaceae [G-2]	
	Peptococcus	
	Peptostreptococcaceae XI [G-1]	
	Peptostreptococcaceae XI [G-10]	
	Peptostreptococcaceae XI [G-11]	
	Peptostreptococcaceae XI [G-13]	
	Peptostreptococcaceae XI [G-2]	
	Peptostreptococcaceae XI [G-3]	
	Peptostreptococcaceae XI [G-4]	
	Peptostreptococcaceae XI [G-5]	
	Peptostreptococcaceae XI [G-8]	
	Peptostreptococcaceae XIII [G-1]	
	Peptostreptococcaceae XIII [G-2]	
	Peptostreptococcus	
	Petrimonas	
	Porphyromonas	
	Propionibacterium	
	Propionivibrio	
	Proteocatella	
	Pseudoclavibacter	
	Schwartzia	
	Sphingomonas	
	SR1	
	Staphylococcus	
	Stenotrophomonas	
	Streptococcus	
	Tannerella	
	Tissierella	
	TM7	
	Treponema	
	Wolinella	
	Xanthomonadaceae [G-1]	
	Xenophilus	

3.3 Variables of interest that influence the opportunity for cross-species transmission of oral bacteria between humans and felines

Initially 20 participants were enrolled in the study. Each participant completed a questionnaire (Appendix A) and the descriptive analysis of the results from the questionnaire was based on all 20 responses. Unlike the need to exclude three participants from data analysis in objectives 1 and 2 due to low samples collection, all 20 completed questionnaires were assessed. The questionnaire questions were a combination of dichotomous, multiple choice (single answer and multiple answers), Likert scale and open-ended questions to assist in identifying any human factors potentially influencing the oral microbiota and the factors that may influence the oral cross-species transmission between humans and felines.

3.3.1 Study participant characteristics

Table 10 summarizes the characteristics of each participant based on age, gender, presence of tooth resorption, type of tooth resorption, number of tooth resorptions, date of diagnoses of tooth resorption, health and dental history, current medications and contact with cats and/or other animals.

Table 10: Study participant characteristics.

	AGE (Y)	GENDER	NO. OF RESORPTIVE TOOTH LESIONS	TYPE OF RESORPTIVE TOOTH LESION	DATE OF DIAGNOSIS	OTHER DENTAL HISTORY	MEDICAL CONDITIONS INDICATED (PAST OR CURRENT)	CURRENT MEDICATIONS	PETS OR FREQUENT CONTACT WITH PETS
EXPERIMENTAL GROUP									
1	> 65	F	1	External	2018	Cavities, root canal, bridge, extraction	Mental health disorder	Citalopram, metoprolol, aspirin, glucosamine sulfate, vitamin D	Frequent contact with cats
2	45–54	M	3	External	2019	Gum disease, root canal	High blood pressure	Allopurinol, amlodipine besylate	No
3	> 65	M	1	Internal	2020	Cavities	High blood pressure, Thyroid disease	Metoprolol, rosuvastatin, losartan, levothyroxine	No
4	55–64	F	1	External	2019	Cavities, root canal	Thyroid disease	Levothyroxine, zopiclone, vitamin D, vitamin C	No
5	45–54	F	2	Internal	2019	Cavities	–	–	No
6	45–54	M	1	Internal	2019	Cavities, root canal	Mental health disorder	–	Cat
7	55–64	M	1	External	2019	Cavities, root canal	–	Tamsulosin, vitamin D	Cat and dog
8	25–34	M	1	External	2019	Root canal	Asthma	–	Cats
9	18–24	M	1	Internal	2015	Braces, dental implant	–	–	Cat
10	45–54	F	2	External	2015	Cavities, root canal, tooth trauma, braces	Immune disorders	Paroxetine, stress probiotic, Adrenal Px Balance, naproxen, vitamin B12	Cats
CONTROL GROUOP									
11	> 65	F	0	N/A	N/A	Gum disease, cavities, root canal	–	–	Cat
12	> 65	M	0	N/A	N/A	Gum disease, cavities	High blood pressure	Blood pressure medications, prostate medication	Cat
13	18–24	M	0	N/A	N/A	Cavities, braces	Seizures	–	No
14	45–54	M	0	N/A	N/A	Gum graft	–	Azathioprine	Cat
15	25–34	M	0	N/A	N/A	–	High blood pressure	Warfarin, metoprolol, aspirin, pantoprazole, ramipril	No
16	55–64	F	0	N/A	N/A	Gingivitis	High blood pressure	Latanoprost, perindopril erbumine	No
17	55–64	M	0	N/A	N/A	Cavities, wisdom tooth extraction	–	–	No
18	45–54	F	0	N/A	N/A	Cavities, root canal, braces, night guard	Shingles	–	No
19	45–54	M	0	N/A	N/A	Night guard	High blood pressure, Stroke, Immune disorders, Cancer	Acetaminophen, nifedipine, chlorthalidone, aspirin	Dog
20	45–54	F	0	N/A	N/A	Cavities, root canal, tooth trauma	–	–	Dog

3.3.2 Method to analyze descriptive data

The first step to analyzing the descriptive data was to collapse and remove as many of the columns where there were clearly too many different responses with too few counts of each for statistical analysis to be performed. For example, the brand of mouthwash used, and the health conditions of the participants were removed. As another example, where there were answers for each of the participant's cats (cat 1, 2, and 3, etc.), an average data value was calculated across all of the cats. Once the data was cleaned up in this manner, it was then ready for analyses.

When analyzing survey categorical data such as (e.g., male vs. female, groups coded into categories such as "1" for "visits dentist twice a year" and "2" for "visits dentist once per year" etc.), the Chi-square test is usually carried out when looking for associations between two categorical variables, whereas log-linear analysis is usually carried out for three or more categorical variables.

However, both Chi-square and log-linear analysis can only be robustly performed if the data meet certain assumptions, and in the case of this study, the following assumption was always violated: more than 80% of the expected cell counts must be > 5 in value. Because the data failed these assumptions, the Fisher Exact Test was used for each of the associations discussed below. NCSS reported the Fisher Exact Test result where only 2 categorical variables were involved. Where the data formed a 2×3 table, the VassarStats online suite of tools was used (http://vassarstats.net/) to perform the Freeman–Halton extension of the Fisher Exact Test.

All these analyses assumed that the participants were only selected into one of the two groups (control or experimental) based on their history of tooth resorption and that pet ownership, age, etc., were not selected for (i.e., all other variables were random).

3.3.3 Relevant questions from the descriptive data

Looking at the cleaned up descriptive data, the following questions seemed most relevant:

1. Is there any association between the number of tooth lesions and whether the study participant has a cat or frequent contact with cats? Or perhaps pets in general?
2. Is there any association between the number of tooth lesions and participant age?
3. Is there any association between the number of tooth lesions and participant sex?
4. Do humans with tooth resorption (experimental group) have worse oral hygiene than those without tooth resorption (control group)?

3.3.3.1 Question 1: Is there any association between the number of tooth lesions and whether the study participant has a cat or frequent contact with cats? Or perhaps pets in general?

This question was analyzed in several ways. First, any association between having a cat as a pet and the participant groups (control vs. experimental) was checked. This is confirming the assumption that the participants were chosen randomly with respect to whether they had cats or not. There was no significant association (Fisher Exact $P > 0.05$).

Second, any association between having a cat as a pet and/or frequent contact with cats and the participant groups (control vs. experimental) was checked. Again, this is confirming the assumption that the participants were chosen randomly with respect to whether they had cats or not. There was no significant association (Fisher Exact $P > 0.05$).

Third, any association between having a cat and/or dog as a pet and/or frequent contact with cats and the participant groups (control vs. experimental) was checked. Again, this is confirming the assumption that the participants were chosen randomly with respect to whether they had pets or not. There was no significant association (Fisher Exact $P > 0.05$).

Fourth, the association between the experimental group, individuals with ERL or IRL, and having a cat was checked. There was no significant association (Fisher Exact Test P > 0.05).

Fifth, instead of dividing the data into control vs. experimental, it was divided into the number of lesions present: 0 lesions, 1 lesion, 2+ lesions. The association between having a cat and/or dogs as a pet and/or frequent contact with cats and the number of lesions was checked. There was no significant association (Figure 13 Freeman–Halton extension of the Fisher Exact Test P > 0.05).

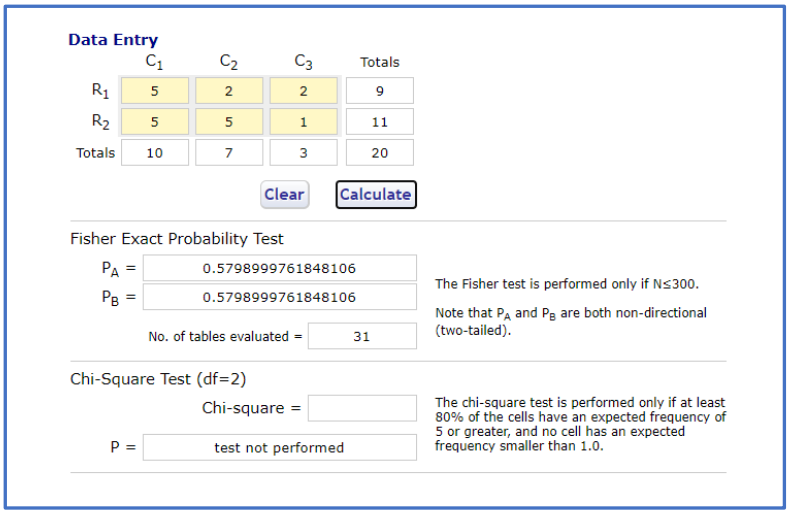

Figure 13: Freeman–Halton extension of the Fisher Exact Test P > 0.05
Association between pets and lesions:
C1 = no lesions, C2 = 1 lesion, C3 = 2 or more lesions
R1 = No cats or dogs or frequent contact with cats
R2 = Have cats or dogs or frequent contact with cats

3.3.3.2 Question 2: Is there any association between the number of tooth lesions and participant age?

Using the data divided into the number of lesions present, any association between participant age and the number of lesions was checked. There was no significant association P>0.05 (Figure 14).

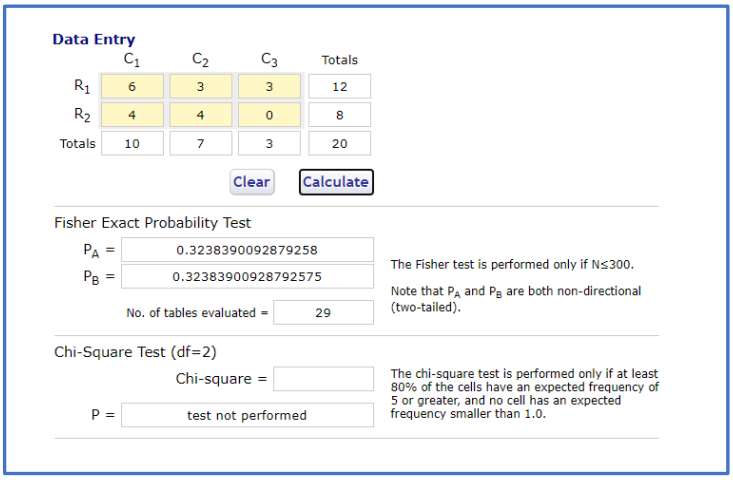

Figure 14: Freeman–Halton extension of the Fisher Exact Test P > 0.05
Association between age and lesions:
C1 = no lesions, C2 = 1 lesion, C3 = 2 or more lesions
R1 = Younger study participants (Coded age groups 1 to 4)
R2 = Older study participants (Coded age groups 5 and 6)

3.3.3.3 Question 3: Is there any association between the number of tooth lesions and participant sex?

Using the data divided into the number of lesions present, any association between participant sex and the number of lesions was checked. There was no significant association P>0.05 (Figure 15).

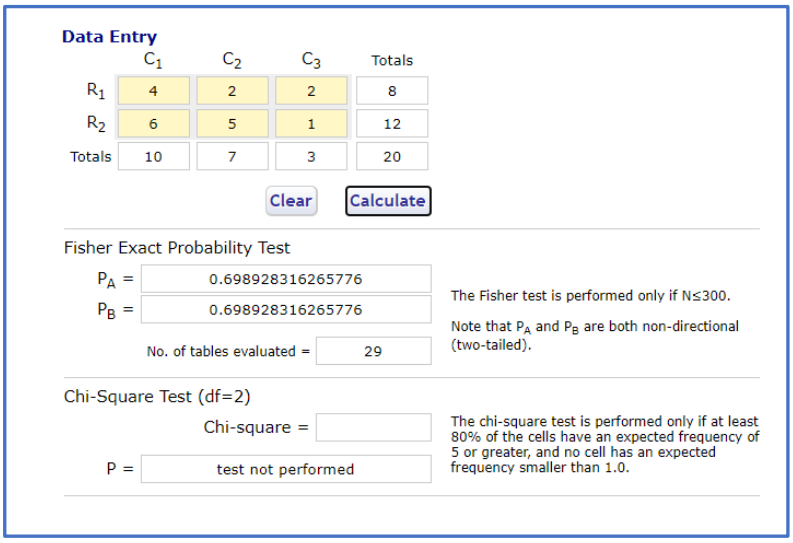

Figure 15: Freeman–Halton extension of the Fisher Exact Test P > 0.05
Association between sex and lesions:
C1 = no lesions, C2 = 1 lesion, C3 = 2 or more lesions
R1 = Female study participants
R2 = Male study participants

3.3.3.4 Question 4: Do humans with tooth resorption (experimental group) have worse oral hygiene than those without tooth resorption (control group)?

A weighted "oral hygiene" score for each participant was calculated from all questions relating to the participants' oral hygiene practices. Lower oral hygiene scores indicated better hygiene practices. The T-test on the experimental vs. the control group scores was performed but there was no significant difference (T-test P > 0.05).

Chapter 4. Discussion

This mixed method case control study assessed: 1. the oral microbiome of humans with tooth resorption and compared it to the oral microbiome of humans without tooth resorption, 2. the oral microbiome of humans with tooth resorption and compared it to the feline oral microbiome and 3. the association between human tooth resorption and contact with felines. To the best of my knowledge this is the first study investigating the oral microbiome of humans with tooth resorption and contact with felines. The overall results of the study indicate that there is no significant difference in the oral microbiome of humans with and without tooth resorption; the human oral microbiome and the microbiome of cats with and without tooth resorption is highly similar with three bacterial genera being similar in the human oral microbiome and the oral microbiome of felines with tooth resorption (*Alysiella*, *Prevotella* and *Rothia*); and there are no significant differences between the association of tooth resorption and contact with felines.

4.1 Study Implications

When comparing the oral microbiome of humans with resorptive tooth lesions to matched controls for approximate age and gender without resorptive tooth lesions, taxonomic composition, differential abundance testing, alpha diversity and beta diversity results all demonstrated that there were no significant differences between the two groups.

A healthy oral microbiome is in a state of equilibrium however when oral disease is present, we see a shift in the balance of oral bacteria (34,35). Pathogenic bacteria become dominant species in the presence of oral disease and the microbial shift to oral disease is known as oral dysbiosis (35). The oral microbiome of humans with periodontal disease and caries has been well studied and it has been shown that there are specific bacteria associated with each of the fore mentioned oral diseases (34,35). There is also evidence to suggest that it is the alpha diversity,

relative number and abundance of species, which can influence disease (35). This is information that has come from years of research and studying these oral diseases. The widespread prevalence of these oral diseases makes studying them less complicated (35–37). With no previous oral microbiome research on tooth resorption in humans and the low prevalence of these lesions, this pilot study has attempted to fill that gap. With a small sample size though, it is difficult to achieve statistically significant results ($p > 0.05$), but inferences can be made using what we know about oral microbiome dysbiosis that further research might show a more pronounced shift in the oral microbiome of humans with ERL and IRL.

With ERL and IRL, there are very few clinical signs or symptoms that would be visible to the patient or clinician which make them difficult to diagnosis (2,9). By the time tooth resorptive lesions are either clinically or radiographically visible, there is usually extensive damage to the tooth (2,9). At that point the tooth is often unrestorable and requires extraction or extensive, expensive dental treatment and long-term, the tooth remains compromised (2,9). With periodontal disease and caries however, there are clinical signs and symptoms which can be assessed to make a diagnosis including oral bacterial testing (37). Often those clinical assessments can be done early and before the chance for the disease to progress (37). The results of this study do not reveal a unique oral microbiome for humans with ERL or IRL. Not knowing if there are bacterial species or species abundance differences in the oral microbiome of people with tooth resorption, prevents the possibility of an earlier diagnosis at a less damaging stage.

Due to the lack of statistical significance between the oral microbiome of the controls and experimental groups, a comparison was done between all human oral microbiome study samples and the feline oral microbiome results from the Thomas et al and the Dewhirst et al studies (23,33). Overall, it was found that humans and felines share a similar oral microbiome. There

were 18 common oral microbiome genera between humans and felines; pathogenic and non-pathogenic. These findings support the theory that it may not be the differences in the oral microbiomes between humans and felines that influence the develop of resorptive tooth lesions, but the similarities.

Even though cats have a higher prevalence of FORL than humans (19), the Thomas study is the first of its kind looking at the oral microbiome of cats with FORL (23). The Thomas et al study did not find any specific bacteria associated with FORL but did identify one subgroup of cats with FORL who had an altered microbiota in comparison to the orally healthy cats and compared to a second subgroup of cats with FORL suggesting that a shift in feline microbiome may be enough trigger virulence and inflammation and result in the development of FORL in susceptible cats (23).

When comparing the human oral microbiome study results to the oral microbiome of cats with FORL from the Thomas et al study, three common genera were identified: *Alysiella, Prevotella* and *Rothia* (23). The average abundances of these genera in the cases and controls of the current study were very similar with *Rothia* being the second most abundant genus in both humans with and without tooth resorption (11.9% and 13.3% respectively). *Rothia* is a facultative anaerobic gram positive rod found in healthy and diseased mouths and the *R dentocariosa* species has been linked to infective endocarditis in immunocompromised patients with poor oral hygiene (38,39). The study survey results from the ten cases are not able to provide statistically significant results to imply that an individual with poor oral hygiene and who is medically compromised is more likely to develop tooth resorption. It is interesting to note though that out of the ten study cases with tooth resorption, four reported being affected by at least one systemic disease and one had an immune disorder. The study participant with an

immune disorder had two resorptive tooth lesions and was a cat owner. As a common and more abundant species in humans and cats with FORL and its association with other oral diseases and systemic infections, it is possible that the presence of *Rothia* may be involved in the virulence and development of tooth resorption in humans and felines (23,38,39).

The number of samples in each group was very low, which limited the descriptive statistical analyses that could be performed. There was no statistically significant association between the presence or number of the participants' tooth lesions and having cats (and/or dogs or frequent contact with cats). Similarly, there was no significant association between the number of the participants' tooth lesions and their age or sex. Finally, a weighted oral hygiene score was calculated for all study participants, but no statistically significant difference was found in this score between the experimental and control groups. These descriptive results loosely imply that any individual at any age, of any gender, with poor or good oral hygiene habits and with or without contact with cats or other animals may be at risk for ERL or IRL.

Because the oral microbiomes of humans with tooth resorption and cats with tooth resorption have not been well studied, there is no previous research to make direct comparisons to the current study. The current study implies there are no connections between human tooth resorption and cat ownership or contact with cats. However, research has shown that people who have pets share a similar oral microbiome to their owners (22,40). Research has also reported the transmission bacterial pathogen associated with dental caries, from caregiver to child (vertical) or from the environment (horizontal)(36). It is sensible to infer then that the opportunities for the transmission of oral bacteria between pets and humans exist and pathogenic oral bacteria typically found in pets, could initiate a shift in the oral microbiome of humans to a state of dysbiosis and the triggering of a disease response.

One Health speaks to this very idea that human health is influenced by the health of our environment and the health of the animals we share our environment with (41,42). Medical issues are becoming more complex, are multifactorial and involve species of non-human origin (41,42). Looking at health concerns collaboratively through medical, veterinary, and ecological science lenses, may fill existing knowledge gaps and improve health for all (41,42).

4.2 Study Limitations & Future Directions

The study's biggest limitation was the small sample size. With no previous research in this area, it was challenging to determine the parameters necessary for a sample size calculation (43,44). However, pragmatic considerations and logistics (time to recruit, costs, etc.) suggested that a sample size of 15 cases (patients with resorptive lesions) and 15 controls (patients selected to match approximately for age and sex) would both be practicable and provide adequate information to inform future research in this area (43,44). Because this study was interrupted by COVID19, the sample size was further reduced to ten cases and ten controls. The small sample size numbers may have greatly impacted the results as there were no significant results in this study from the quantitative or qualitative data. Continuing to study the oral microbiome of people with tooth resorption to identify a specific oral microbiome and increasing the sample size would hopefully decrease margins of error and improve power to get a statistically significant result.

Ownership and/or significant contact with cats was not an inclusion criterion for study participants (cases or controls) because of the low prevalence of resorptive lesions and including cat ownership and/or cat contact would have further decreased the sample size. The participant questionnaire identified cat ownership and/or contact with cats however a future study

consideration would be to only include participants with tooth resorption who have cats. Oral samples could then be taken from both the human participant with tooth resorption and their cat to make a comparison between the two. Further collaboration with a veterinarian to diagnosis the oral health of the participants' cat and confirm the presence of FORL would have beneficial outcomes for identifying bacteria associated with tooth resorption.

The oral samples taken for the experimental and control groups were from supra gingival areas of the teeth. Studies looking at the oral microbiome have shown differences in detectable species when taking saliva samples, supra gingival and sub gingival plaque samples (34,45,46). This type of sampling could have impacted study results as more diversity could have been found between the control and experimental groups with sub gingival sampling.

The feline oral microbiome database from the Dewhirst study took plaque samples from the sub gingival area (33). The current study took supra gingival plaque samples. Comparing the human supra plaque samples to the sub gingival feline oral samples creates an obvious discrepancy.

Tooth resorption can be stimulated by dental procedures such as endodontic treatment, orthodontic treatment, intracoronal bleaching (non-vital tooth bleaching), periodontal treatment and trauma (2,7,12). One study looking at the factors associated with external cervical tooth resorption found the majority of cases with tooth resorption were multifactorial (7). This study also reported associations of poor oral health, parafunctional habits (eg bruxism & nail biting), systemic health and viral infections with tooth resorption (7). The fact that some case participants indicated they had previous orthodontic or endodontic treatment may have been an influential factor to the development of their tooth resorptive lesions. A more detailed

investigation into the medical and dental histories of individuals with tooth resorption in the future studies is necessary, as to prevent any confounding effects on the results.

Overall, more studies are needed in this underdeveloped area of research looking at both the oral microbiome of humans ERL and IRL and cats with FORL. Once specific pathogenic tooth resorptive lesion bacteria are identified, the next step would be the look at the mechanisms of action and how the pathogenic bacteria or shift in taxonomic composition of bacteria trigger the initiation of a tooth resorption lesion.

4.3 Conclusion

In conclusion, this study suggests that humans with tooth resorption do not have a unique oral microbiome in abundancies or species. Given the fact that there is such high interindividual variation in both the human and cat oral microbiomes, with conservation at the phylum rather than species level, it would be unlikely to observe a significant difference in the oral microbiomes between humans (with or without tooth resorption) and felines. There is no evidence to suggest that owning a cat or pet influences the probability of developing tooth resorptive lesions. The study limitations do indicate that further research is needed in this area as the implications to better understanding the oral microbiome of people with tooth resorption may help diagnose this oral disease earlier preventing more destructive damage. Further exploration is needed on the area of oral health and its connection to the transmission of oral bacteria from household pets. The health of our environment and the health of the people and animals we share our environment with may provide us with answers to our unanswered oral health questions and concerns (41,42).

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Appendix A: Participant Questionnaire

Please answer all questions to the best of your ability. All information is for the purposes of the study only, it will be kept confidential, stored securely, and no information will be passed on or shared.

1. What is your age? (check one)
    - 18-24
    - 25-34
    - 35-44
    - 45-54
    - 55-64
    - 65 & older

2. What is your gender? (check one)
    - Male
    - Female
    - Other

3. Please check to indicate if you have or have had any of the following conditions (check all that apply):

○ High blood pressure	○ Thyroid disease
○ Diabetes	○ Mental health disorder (i.e. depression, anxiety, etc)
○ Asthma	○ Seizures
○ Heart attack	○ Immune disorders (i.e. rheumatoid arthritis, lupus, etc)
○ Stroke	○ Stomach or bowel conditions (i.e. irritable bowel syndrome, Crohn's disease, colitis, etc)
○ Osteoporosis	○ Cancer
○ Kidney disease	○ Genetic disorder
○ Shingles	○ Other: _____

4. List all medications:

    _____

    _____

    _____

5. How often do you have dental check-ups? (check one)
    - Less than once per year
    - Once a year
    - Twice a year
    - More than twice a year
    - Other: _____

6. How often do you have dental hygiene cleanings? (check one)
    - Less than once per year
    - Once a year
    - Twice a year
    - More than twice a year
    - Other: _____

7. What is your daily routine for taking care of your teeth:
    a. Do you brush your teeth?

        - Yes
        - No

        - If yes to question 7a, how often do you brush your teeth? (circle answer)

    2 times/day once/day 2-5 times/week once/week less than once/week

        - If yes to question 7a, do you use brush with toothpaste with fluoride? (circle answer)

    Yes No Don't Know

        - If yes to question 7a, do you brush with a sensitivity toothpaste? (circle answer)

    Yes No Don't Know

    b. Do you clean in between your teeth? (ex. Flossing, toothpicks, proxabrushes, etc)

        - Yes
        - No

        - If yes to question 7b, how often do you clean in between your teeth? (circle answer)

    2 times/day once/day 2-5 times/week once/week less than once/week

c. Do you rinse with mouthwash?

- o Yes
- o No

- If yes to question 7c, how often do you rinse your mouth? (circle answer)

2 times/day once/day 2-5 times/week once/week less than once/week

- If yes to question 7c, what brand of mouth rinse do you use? (check all that apply)
  - o Listerine
  - o Crest ProHealth
  - o Colgate
  - o Scope
  - o Prescription
  - o Other: _____

8. Do you have or have you had any of the following dental conditions or treatments? (check all that apply)
   - o Gingivitis
   - o Gum disease
   - o Cavities
   - o Root canal
   - o Tooth trauma or accident
   - o Braces
   - o Night guard
   - o Other: _____

9. How many resorptive tooth lesions do you have? (check one)
   - o 1
   - o 2
   - o 3
   - o 5 or more
   - o Don't know

10. Have you been diagnosed with a resorptive tooth lesion before? (check one)
    - o Yes
    - o No

11. Do you have a cat in your home? (check one)
    - o Yes - continue with question 12
    - o No - go to question 25

12. How many cats do you have? (check one)
    - o 1
    - o 2
    - o 3
    - o 4
    - o 5 or more

13. How long has the cat(s) lived in your home? (circle one answer for each cat in the home)

Cat 1: less than 1 year	1-5 years	5-10 years	over 10 years
Cat 2: less than 1 year	1-5 years	5-10 years	over 10 years
Cat 3: less than 1 year	1-5 years	5-10 years	over 10 years
Cat 4: less than 1 year	1-5 years	5-10 years	over 10 years

14. What is the age of the cat(s)? (circle one answer for each cat in the home)

Cat 1: 0-1 years	old	1-5 years old	5-10 years old	over 10 years old
Cat 2: 0-1 years	old	1-5 years old	5-10 years old	over 10 years old
Cat 3: 0-1 years	old	1-5 years old	5-10 years old	over 10 years old
Cat 4: 0-1 years	old	1-5 years old	5-10 years old	over 10 years old

15. Is the cat an indoor cat, outdoor cat(s) or both? (circle one answer for each cat in the home)

Cat 1: indoor	outdoor	both
Cat 2: indoor	outdoor	both
Cat 3: indoor	outdoor	both
Cat 4: indoor	outdoor	both

16. Does the cat(s) eat wet food, dry food or both? (circle one answer for each cat in the home)

Cat 1: wet food	dry food	both
Cat 2: wet food	dry food	both
Cat 3: wet food	dry food	both
Cat 4: wet food	dry food	both

17. On a scale of 1-10 with 1 being very poor and 10 being very healthy, how to you rate the overall health of your cat(s)? (circle one number for each cat in the home)

1 very poor health — 10 very healthy

Cat 1:	1	2	3	4	5	6	7	8	9	10
Cat 2:	1	2	3	4	5	6	7	8	9	10
Cat 3:	1	2	3	4	5	6	7	8	9	10
Cat 4:	1	2	3	4	5	6	7	8	9	10

18. How often does the cat go for veterinary care? (circle one answer for each cat in the home)

Cat 1:	Less than 1x/year	1x/year	2x/year	More than 2x/year
Cat 2:	Less than 1x/year	1x/year	2x/year	More than 2x/year
Cat 3:	Less than 1x/year	1x/year	2x/year	More than 2x/year
Cat 4:	Less than 1x/year	1x/year	2x/year	More than 2x/year

19. Are the cat's immunizations up to date? (circle one answer for each cat in the home)

Cat 1:	Yes	No
Cat 2:	Yes	No
Cat 3:	Yes	No
Cat 4:	Yes	No

20. Has your cat been diagnosed with any medical conditions? (circle one answer for each cat in the home) If yes, list for each cat in the home:

Cat 1:	Yes	No	If yes, list:_____
Cat 2:	Yes	No	If yes, list:_____
Cat 3:	Yes	No	If yes, list:_____
Cat 4:	Yes	No	If yes, list:_____

21. Do you brush your cat's teeth? (circle one answer for each cat in the home)

Cat 1:	Yes	No
Cat 2:	Yes	No
Cat 3:	Yes	No
Cat 4:	Yes	No

22. Has your cat been diagnosed with any dental conditions? If yes, circle all that apply for each cat in the home:

Cat 1: gingivitis	gum disease	tooth loss	tooth resorption	other: _____
Cat 2: gingivitis	gum disease	tooth loss	tooth resorption	other: _____
Cat 3: gingivitis	gum disease	tooth loss	tooth resorption	other: _____
Cat 4: gingivitis	gum disease	tooth loss	tooth resorption	other: _____

23. What type of contact do you have with cat? (Check all that apply for each cat in the home)

Cat 1:

○ petting cat	○ cat sharing your food	○ washing cats eating or drinking bowl with your dishes
○ cat licking you	○ cat sleeping with you	○ cat drinking from bathroom or kitchen sink
○ cat scratches you	○ nuzzling with cat	○ cat eating or drinking off dishes or utensils used by you

Cat 2:

○ petting cat	○ cat sharing your food	○ washing cats eating or drinking bowl with your dishes
○ cat licking you	○ cat sleeping with you	○ cat drinking from bathroom or kitchen sink
○ cat scratches you	○ nuzzling with cat	○ cat eating or drinking off dishes or utensils used by you

Cat 3:

○ petting cat	○ cat sharing your food	○ washing cats eating or drinking bowl with your dishes
○ cat licking you	○ cat sleeping with you	○ cat drinking from bathroom or kitchen sink
○ cat scratches you	○ nuzzling with cat	○ cat eating or drinking off dishes or utensils used by you

Cat 4:

○ petting cat	○ cat sharing your food	○ washing cats eating or drinking bowl with your dishes
○ cat licking you	○ cat sleeping with you	○ cat drinking from bathroom or kitchen sink
○ cat scratches you	○ nuzzling with cat	○ cat eating or drinking off dishes or utensils used by you

24. How often do you have the above contact with the cat? (circle one answer for each cat in the home)

Cat 1: Almost Always	Sometimes	Every once in a while	Rarely	Never
Cat 2: Almost Always	Sometimes	Every once in a while	Rarely	Never
Cat 3: Almost Always	Sometimes	Every once in a while	Rarely	Never
Cat 4: Almost Always	Sometimes	Every once in a while	Rarely	Never

25. If you don't own a cat, do you have contact with cats outside of your home? (check one)

    - Yes
    - No
    - If yes, how often is the contact? (circle one answer)

Almost Always Sometimes Every once in a while Rarely Never

26. Do you have any other pets? (Check all that apply)
    - Dog
    - Bird
    - Hamster
    - Rabbit
    - Other: _____

Thank you. This is the end of the questionnaire.