Chronic Stomatitis in Cats: Clinical Implications of Metagenomic Analysis of the Oral Microbiome

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Background & Rationale

Feline stomatitis is a challenging disease to manage, and there is no gold standard plan for treatment. Currently, clinicians use a trial and error method that usually consists of routine teeth cleaning, extractions, and chronic antimicrobial and corticosteroid therapy, depending on the severity of each case. Unfortunately, even with prudent treatment, many cases of feline stomatitis become chronic, and finding the exact etiology can be difficult. Antimicrobial therapy is included in most management plans, but bacterial culture and sensitivity testing is not part of most diagnostic protocols upon initial presentation. Cats usually present in the clinic with signs of oral pain, including anorexia and dysphagia, and the top priority is to resolve these issues to help the cat eat independently. However, empirical treatment of feline stomatitis with amoxicillin, amoxicillin-clavulanate, clindamycin, or metronidazole without proper diagnostic information can promote bacterial resistance and be ineffective in the treatment of this disease. Cultures are expensive, time consuming, and less sensitive as compared to next-generation sequencing. The MinION, a new device by Oxford Nanopore Technologies, is a portable sequencer that provides real-time analysis of DNA and RNA from complex samples. The technology relies on passage of DNA or RNA strands through nanopores, which disrupts an ionic current that allows for reading of nucleotide bases, thus providing an accurate and extensive picture of the microflora present. Additionally, the technology can identify plasmids, thus providing information on antibiotic resistance and guide the choice of antimicrobial treatment. MiDOG (Irvine, CA) can provide comparable data using Illumina technology, which is the focus of this poster presentation. Previous studies on cats with stomatitis have examined severity of oral lesions in reference to Feline Calicivirus infection, the microflora of cats infected with Feline Immunodeficiency Virus versus clinically healthy controls, and assessment of control cats with healthy gingiva compared to cats with gingivitis and mild periodontitis (not stomatitis). In contrast, this pilot study focused on cats with stomatitis that have been vaccinated, tested, or assumed to be virus-free based on presentation, when generating the oral microbiome.

Hypothesis & Objectives

Hypothesis. The Illumina (or MinION) sequencer will provide a more complete and accurate picture of the microflora in feline stomatitis, thus elucidating on optimal antimicrobial choice and providing superior insight on the underlying pathology.

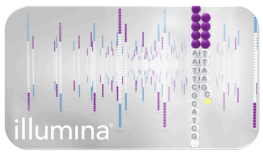
Objectives. (1) Obtain oral swabs from clinically healthy cats vs. patients diagnosed with stomatitis. (2) Use the Illumina (or MinION) technology to compare oral microbiome in cats with stomatitis vs. controls. (3) Elucidate on antimicrobial resistance plasmids in the feline oral microbiomes using MinION/Illumina. (4) Use MinION/Illumina data to guide antimicrobial therapy in stomatitis patients.

Design & Methods

The study assessed two groups: five (5) clinically healthy cats (controls), and two (2) cats diagnosed with chronic stomatitis. Clients signed a consent form approved by the WesternU Institutional Animal Care and Use Committee (IACUC). Oral swabs were collected from each cat and DNA amplified using an Illustra Ready-To-Go GenomiPhi V3 DNA Amplification Kit. The oral microbiome was established by MiDOG, LLC using Illumina technology (and will subsequently be established at WesternU using MinION technology from Oxford Nanopore Technologies, as a related study). Bacterial taxonomy and resistance plasmids were recorded and compared between the two groups.

1. Oral swab collection from cats

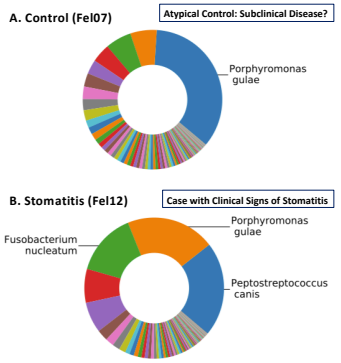
2. DNA Isolation & Amplification



3. Metagenomic analyses (Illumina & MinION)

Note: metagenomic data from feline oral swabs will also be generated using the *handheld* MinION device from Oxford Nanopore Technologies, and data will be compared with the Illumina data. Again, this poster presentation will only focus on the metagenomic data generated using Illumina (which is a much larger *bench-top sized* piece of laboratory equipment (i.e., not portable like the MinION). As such, the MinION technology is also of immediate interest for broader clinical use in feline medicine.

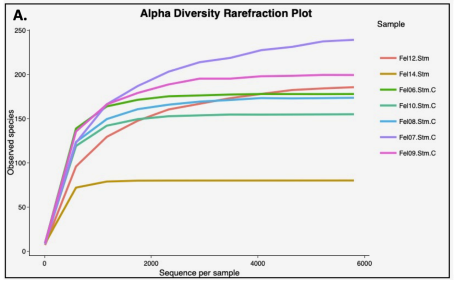
Results



Species Detected in ONE Clinically Healthy Control Cat (Fel07)	Relative Abundance
Porphyromonas gulae	33.8%
Bacteroides pyogenes	5.93%
Fusobacterium sp.	5.78%
Alloprevotella sp.	4.22%
Moraxella sp.	3.18%
Bacteroides denticanum	2.98%
Lampropedia sp.	2.8%
Fusobacterium nucleatum	2.48%
Arcobacter sp.	2.34%
Campylobacter rectus	1.63%
Proteocatella sp.	1.57%
Spirochaetaceae sp.	1.52%
Defluviitaleaceae sp.	1.11%
Capnocytophaga canimorsus	1.04%
Fusibacter sp.	0.91%

Species Detected in ONE Cat with Stomatitis (Fel12)	Relative Abundance
Peptostreptococcus canis	20.5%
Porphyromonas gulae	19.5%
Fusobacterium nucleatum	13.7%
Bacteroides pyogenes	7.35%
Streptococcus intermedius	6.67%
Actinomyces urogenitalis	2.44%
Filifactor villosus	1.86%
Alloprevotella sp.	1.63%
Roseburia sp.	1.46%
Pasteurella dagmatis	0.99%
Fretibacterium sp.	0.95%
Peptococcus sp.	0.92%
Spirochaetaceae sp.	0.76%
Tannerella forsythia	0.72%
Spirochaetaceae sp.	0.72%

Figure 1 – Charts represent relative amount and species of bacteria detected in the oral cavity of *one* (atypical control) clinically healthy cat (A) vs. *one* cat with stomatitis (B). Each color represents a different bacterial species (the larger the size of the colored piece, the more abundant the bacterial species). The tables list species and relative microbial abundance in the oral swabs from the two cats (atypical control (subclinical disease?) vs. cat with stomatitis)



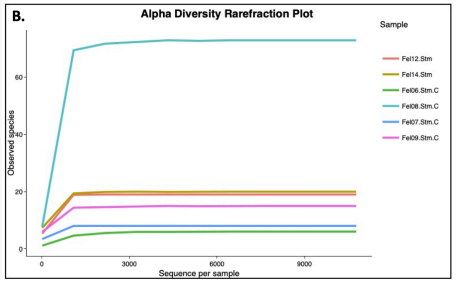
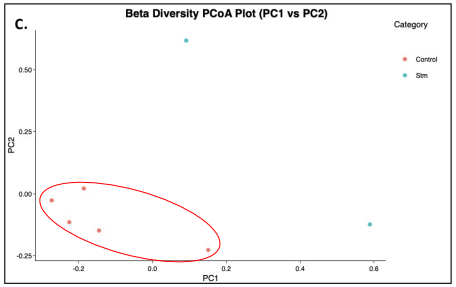


Figure 3 – **Above**: Alpha diversity plots represent *bacterial* (A) and *fungal* (B) *taxonomic diversity* of individual oral swabs from cats with stomatitis versus controls. **Below**: Beta diversity plot (C) reflects Principal Coordinate Analysis (PCoA) based on Bray-Curtis dissimilarity, which serves as a measure of the *microbial structure diversity* between oral swab samples. Here, we noticed a clustering pattern amongst controls (red circle), while the two cats with stomatitis differed widely in oral microbial diversity structure (turquoise).



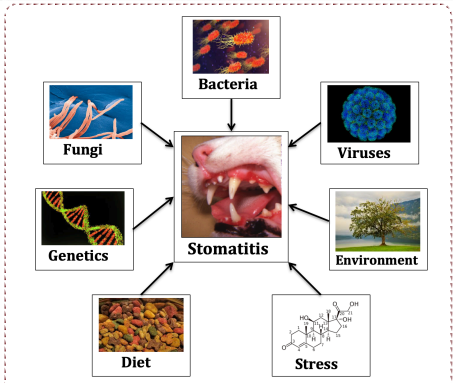


Figure 4 – Schematic showing the multi-factorial nature of feline stomatitis

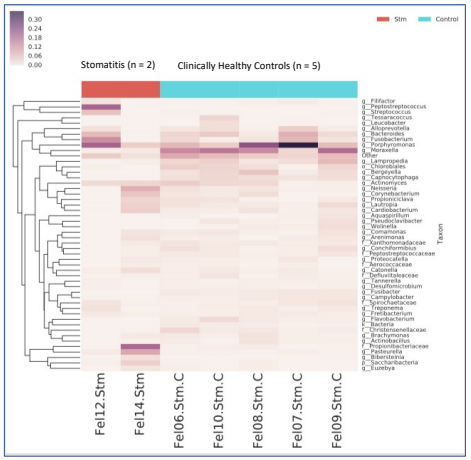


Figure 2 – Taxonomy abundance heat map showing microbial composition in oral swabs from cats with stomatitis (red, n = 2) vs. controls (turquoise, n = 5).

Discussion & Conclusion

This pilot study suggests the feline oral microbiome differs *between* clinically healthy cats (n = 5) and cats with stomatitis (n = 2). However, the data also reflects differences in microbial abundance and diversity *within* groups. Moraxella sp. (associated with healthy feline gingiva) had the highest relative abundance in most controls (Fig.2). Still, the microbial profile of one control (Fel07) was very different given a high abundance of *Porphyromonas gulae* (Fig.1), a bacterium associated with periodontal disease, which might represent subclinical disease. The alpha-diversity plots revealed high levels of *taxonomic diversity* in both clinically healthy cats and stomatitis patients (number of *bacterial* and *fungal* species ranged from 75 to > 225 (Fig.3.A), and 5 to 70 (Fig.3.B), respectively). These are clinically relevant findings, since most species are not detected with conventional testing. The beta-diversity plot (Fig.3.C) reflects *microbial structure diversity*, which suggests a clustering pattern for most controls (red), while cats with stomatitis (turquoise) differ widely. Findings are consistent with a complex multi-factorial disease process (Fig.4), although the low number of cases is a limitation. Clindamycin resistant bacteria were detected in 71% of cats (4 controls, 1 stomatitis), which is a clinically important finding since clindamycin is used routinely for periodontal disease, tooth extraction, oral surgery, and various dental infections, including feline stomatitis. This underscores the justification for metagenomics in feline medicine, plus added insight on cross-species transmission risks in cat bite cases.

Acknowledgements & References

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