

The relationship between periodontal disease of feline and oral core microbiome

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Abstract

The microbiome of the oral cavity is complex, with the average adult harboring about 50 to 100 billion bacteria, which represent about 200 predominant bacterial species. There are a large number of microbes in the oral cavity of the feline, which are normally beneficial to oral health and safety maintenance. However, when the balance of bacteria is lost, feline stomatitis and periodontitis may occur, which seriously endanger local oral health. Environmental implication, feeding model, age, and genetic factors may be the main factors affecting the oral composition of a microbiome. *Filifactor alocis*, *Porphyromonas gingivalis*, and *Tannerella forsythia* and their interactions are the main pathogen that causes feline periodontal disease. In this review, we demonstrated the core microbiome of the feline oral microbe and its effect factors, as well as the connection with periodontal disease.

Keywords: Feline, Oral microbiome, Periodontal disease, pathogenic microorganisms

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Introduction

The oral cavity plays a crucial role in food intake and initial digestion, housing a diverse range of microorganisms. Extensive literature is available describing the oral microbiome that may play a role in oral health. The oral microbiome has been implicated in the oral health of cats and its disease. Imbalances in the subgingival flora often trigger an immune response that leads to periodontal disease.

Periodontal disease is an inflammatory condition that occurs naturally in various mammalian species, including domestic cats and humans (Perry and Tutt, 2015; Marcenés *et al.*, 2013). Cats with periodontitis usually experience symptoms such as halitosis, salivation, pain, loss of appetite, loose teeth, and abnormal tooth loss, which seriously affects the quality of life. Its etiology involves a complex interplay between the microbial community present in the subgingival plaque and the host's immune response (Pan *et al.*, 2019; Silva *et al.*, 2015). Recent molecular studies have shed light on some microbial characteristics of the disease (Rodrigues *et al.*, 2019; Harris *et al.*, 2015; Dewhirst *et al.*, 2015; Sturgeon *et al.*, 2014). For instance, previous studies utilizing 16S rRNA gene sequencing have shown significant differences in subgingival microbial taxonomic profiles between cats with and without periodontal disease (Rodrigues *et al.*, 2019). Several studies have also demonstrated that the microbial composition in cats changes as periodontal tissue transitions from a healthy state to the early stages of disease (Harris *et al.*, 2015), highlighting the close association between the subgingival microenvironment and disease onset and progression. In fact, research has indicated that the functional characteristics of the subgingival microbiome have a more significant impact on host immune responses in humans than its taxonomic composition (Yost *et al.*, 2015; Duran-Pinedo and Frias-Lopez, 2015).

In cats, oral health issues are among the most common health problems (Berg *et al.*, 2020; Alessandri *et al.*, 2020). Dental disease affects approximately 50-90% of cats (Oskarsson *et al.*, 2021). Among dental diseases, periodontal disease is the most prevalent in cats, with varying degrees of severity. Enhancing our understanding of the cat's oral microbiota will undoubtedly contribute to improving their oral health (Harris *et al.*, 2015). Therefore, exploring changes in microbial quantity and species within the microenvironment of cat periodontal disease is a valuable research topic. Numerous studies have established a close relationship between alterations in the composition and abundance of oral microbiota and the occurrence and development of oral and systemic diseases. DNA sequencing technologies and advanced bioinformatics tools are valuable in unraveling the complexity of the subgingival microbiome in both periodontal health and disease (Könönen and Müller, 2014). Understanding the pathogenesis of periodontal disease from a microbial function perspective is crucial for veterinary medical applications and can serve as a foundation for the future development of novel preventive, diagnostic, and therapeutic interventions. These interventions may complement or replace

traditional approaches that have lacked a theoretical basis for decades. This review discusses the relationship between oral microbiota and periodontal disease in cats, providing a theoretical basis and research ideas for utilizing oral microbiota as biomarkers for the prevention, diagnosis, and treatment of the disease.

Oral Periodontal Microbiota in Healthy Cats

Traditionally, pathogenic microorganisms in medical tests have been identified through plate counting. However, this method is time-consuming, laborious, and limited in scope, only able to identify a few microorganisms on a single plate (Whitley, 2008). In contrast, high-throughput sequencing technology, a culture-independent approach, offers high resolution, good repeatability, and the ability to detect nearly all microorganisms in a specific environment, even those with low DNA content or in a dormant state (Wang *et al.*, 2012). Despite recent advancements, there is still a lack of published data on the cat microbiome compared to humans and other animals.

Sturgeon *et al.* (2014) utilized next-generation sequencing technology to analyze the oral microbiota of healthy cats. They found that it exhibited high diversity, richness, and uniformity. The dominant bacteria in healthy cats were *Porphyromonas*, followed by *Moraxella* and *Fusobacterium*. Another study by Dewhirst *et al.* (2015) developed a reference set for the cat oral microbiome using the 16S rRNA gene. Through this method, they identified 11 phyla in the oral microbiome of 248 sampled cats, including *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Spirochetes*, *Actinobacteria*, *Intertrophic Bacteria*, *Chlorobacteria*, *Chlorocurved Bacteria*, *Fusobacteria*, SR1, and TM7. Alder *et al.* (2016) demonstrated that the oral microbiome in cats is comprised of a wide variety of bacteria from 14 phyla. They identified a total of 411 bacterial operational taxonomic units (OTUs) in the oral bacteria of all cats. Most of these OTUs (67%) belonged to genera with an abundance of less than 1%, while the remaining 33% of OTUs had an abundance of more than 1%, accounting for the majority of sequences (76%). *Bacteroides* (31%), *Firmicutes* (24%), and *Proteobacteria* (21%) were the three dominant phyla, collectively representing 76% of the sequences.

In summary, comprehensive findings indicate that the oral microbiota in healthy cats is uniform and stable. The most dominant operational taxonomic unit is *Porphyromonas*, followed by *Moraxella* and *Clostridium*.

Factors Affecting Oral Composition of Microbiome

In a normal physiological state, the oral microbiome and the host typically maintain a delicate balance. Saliva, on the one hand, provides a favorable natural environment for the growth and colonization of diverse microbes in the oral cavity. It

offers suitable conditions such as temperature, humidity, nutrient sources, a peroxidase system, specialized anatomical structures, and specific physical and chemical properties. On the other hand, there are numerous interactions among the oral microbial communities, including nutritional competition, synergy, antagonism, and the presence of virulence factors (Shi and Zhou, 2010). However, the composition of the oral microbiome can be influenced by various factors that impact the saliva environment.

Environmental Implication: Environmental factors play a significant role in shaping the oral microbiome. Research studies have demonstrated the impact of environmental conditions on the composition of oral microbial communities.

In a survey by Stahnger *et al.* (2012), it was observed that the oral microbial community similarity between twins was higher when they lived together compared to when they were separated. This suggests that environmental factors have a substantial influence on the oral microbiome, as the similarity decreased when the twins were exposed to different environments.

Another study investigated the association between multi-cat environments, outdoor access, and the prevalence of feline chronic gingivostomatitis (FCGS) (Peralta and Carney, 2019). The findings revealed that the prevalence of FCGS was higher in households with multiple cats compared to single-cat households. Furthermore, the odds of FCGS increased by approximately 70% with each additional cat in the household. This indicates that the environment, characterized by the presence of multiple cats, can influence the oral microbial community and contribute to the development of oral health conditions in cats.

Overall, the richness and composition of oral microbes can vary significantly in different environments. Environmental factors have a substantial influence on the microbial community and can play a crucial role in shaping the oral microbiome.

Feeding Model: In the study of the influence of environmental factors on the oral microbiome, researchers have specifically investigated the impact of diet on its diversity. Several studies have shown that diet can affect the oral microbiome and its composition.

Huang *et al.* (2011) examined the antibacterial activity of fatty acids against oral microbes and found that fatty acids can influence oral microecology by altering the environment of the gingival sulci and impacting the microbial membrane. They demonstrated that fatty acids have inhibitory effects on oral bacteria.

Johansson *et al.* (2018) discovered an association between high milk intake and the oral microbiome. They found that reducing bacterial species associated with specific diseases, such as *Streptococcus mutans*, through control of milk intake may regulate the development of complex diseases influenced by other factors like sugar intake.

Christina's study (Adler *et al.*, 2016) also revealed the significant effect of diet on species richness in the oral microbiome of cats. Cats fed a dry diet had higher

operational taxonomic unit (OTU) diversity compared to cats fed a wet diet. The study identified *Actinobacillus*, *Acholeplasma*, *Treponema*, and *Porphyromonas* as the most enriched bacteria in cats consuming dry diets. On the other hand, cats consuming wet diets had a significantly higher abundance of *Proteobacteria*, particularly from the *Neisseriaceae* family, including *Conchiformibius kuhniae*.

In summary, diet plays a significant role in shaping the oral microbiota of cats. The type and composition of food consumed can influence the stability and diversity of microbes in the oral cavity.

Age and sex: Age and sex are additional factors that can influence the richness and composition of the oral microbial community in cats.

Studies have shown that the abundance of rare bacteria involved in biosynthesis differs in the oral microbiomes of cats of different ages. For example, a microbial study (Older *et al.*, 2020) found that older cats (13-15 years old) had a higher relative abundance of bacteria associated with l-methionine and L-arginine biosynthesis, while mature cats (7-9 years old) had a higher relative abundance of bacteria related to glucose and xylose degradation. Furthermore, the relative abundance of bacteria involved in l-arginine synthesis was found to be higher in female cats.

Gender has also been found to have a significant effect on the dominant genera of aerobic bacteria in the oral microbiome of cats. A study by Whyte *et al.* (2017) showed that a higher percentage of female cats (70.8%) carried more than two dominant genera of aerobic bacteria, while a higher percentage of male cats (80%) carried two or fewer dominant genera.

Moreover, bacterial diversity has been observed to increase with age in older age groups. In the oral cavity of adult and young cats, the most common genera were *Pasteurella* and *Streptococcus*, followed by *Moraxella* and *Neisseria*. In contrast, in the elderly group, the dominant genus was *Neisseria*, followed by *Pasteurella* and *Staphylococcus* (Sondorová *et al.*, 2021).

These findings indicate that age and sex can impact the oral microbial community in cats, influencing the abundance of specific bacterial groups and contributing to variations in microbial diversity and composition across different age groups.

Genetic factors can also play a role in shaping the oral microbiome in cats: A study by Papapostolou *et al.* (2011) investigated the impact of host genotypes on the composition of subgingival microbiota and found no significant difference in the number of oral microbial species and the similarity of subgingival microbiota shared by identical and fraternal twins. However, other studies have shown that genotypes can influence the diversity and taxonomic composition of the oral microbiome (Tong *et al.*, 2014; Goodrich *et al.*, 2014). Genetic factors have even been associated with certain genetic disorders. For example, specific NOD2 genotypes have been linked to increased oral abundance of *Clostridium difficile* (Li *et al.*, 2012a) and *Enterobacteriaceae* (Knights *et al.*, 2014) in cats at high risk for inflammatory bowel disease.

These findings suggest that genetic factors can contribute to variations in the oral microbiome,

influencing the diversity of microbial species and the taxonomic composition of the community. Genetic predispositions may also play a role in the susceptibility to certain oral health conditions or diseases in cats.

Differences in Oral Microbe Composition between Periodontal Disease and Healthy Cats

Periodontal disease, particularly periodontitis, is characterized by the destruction of periodontal tissue and an increase in periodontal pocket depth (Iwashita et al., 2019; Perry and Tutt, 2015). It is believed that bacteria in the oral biofilm can be released into the periodontal pocket, where they may enter the bloodstream through ulcers in the pocket epithelium or stimulate the production of pro-inflammatory factors and acute phase proteins, leading to systemic inflammation (Dentino et al., 2013). Pro-inflammatory mediators such as IL-1 β and IL-8, as well as markers of tissue destruction like matrix metalloproteinase-8, can be measured in gingival secretions to assess the extent of periodontitis (Teles et al., 2010).

While the exact microbial and immunological mechanisms underlying feline periodontal disease are not fully understood, recent molecular studies have revealed some microbial characteristics of the disease. For instance, Rodrigues et al. (2019) and Perry and Tutt (2015) used 16S rRNA gene sequencing and found that the oral microbiome of cats with periodontitis had lower diversity compared to healthy cats, and there were significant differences in the subgingival microbial taxonomic profiles between the two groups. Dewhirst et al. (2015) identified key pathogens, such as *treponema* and *Porphyromonas gingivalis*, using bacterial culture and DNA sequencing in cats with

periodontitis and other oral diseases. Additionally, *Pasteurella multocida* subspecies were found to be more prevalent in affected cats compared to controls when both culture-dependent and independent methods were used (Dolieslager et al., 2011).

In healthy cats, important microbial communities include *Porphyromonas*, *Moraxella*, *Clostridium*, *Xanthomonadaceae*, *Capnocytophaga canimorsus*, and *Bergeyella* species (Rodrigues et al., 2019; Li et al., 2012b). A study by Sendorová et al. (2021) on the oral microbiota of cats with and without disease showed that *Streptococcus* was the most common genus in the oral microbiota of healthy cats, while *Staphylococcus* was predominant in the oral microbiota of diseased cats. *Spirochetes* and *Bacteroidetes* are major pathogens associated with oral diseases in cats, such as gingivitis and mild periodontitis (Karatas and Gevrek, 2021). Another study found that they were common in infected cats (Dolieslager et al., 2011). Several studies, including those by Dolieslager et al. (2011) and Perez-Salcedo et al. (2011), have concluded that the diversity of the oral microbiota is lower in diseased cats compared to healthy cats. *Pasteurella multocida* was found to be prevalent in both healthy and diseased cats. Table 1 provides a comparison of dental plaque bacteria between healthy and diseased cats.

In comparison to healthy cats, periodontitis is associated with a significant increase in the abundance of bacteria in *Spirulobacteria* and *Firmicutes*, while the abundance of bacteria in *Proteobacteria* and *Fusobacteria* decreases significantly. Therefore, periodontitis is considered a complex microbial infection characterized by an increase in potentially pathogenic microorganisms in the oral microbiome, which can trigger inflammatory responses and alter the nutritional environment of the oral microbiome, ultimately leading to the development of the disease.

Table 1 Comparison of dental plaque bacteria in healthy cats and cats with oral diseases

Dominant microbiota in cats with oral diseases	Dominant microbiota in healthy cats	Reference
<i>Chordata, Spirochaetes</i>	<i>Actinobacteria, Proteobacteria</i>	Perry et al. (2015)
<i>Treponema, Peptostreptococcus</i>	<i>Enhydrobacter, Moraxella, Capnocytophaga sp., Bergeyella, Comamonas, Dichelobacter, Actinobacillus, Myroides</i>	Dolieslager et al. (2011)
<i>Porphyromonas gulae, Porphyromonas circumdentaria, Fusobacterium nucleatum</i>	-	Pérez-Salcedo et al. (2011)
<i>Enterococcus faecalis, Bacteroides</i>	<i>Pasteurella multocida</i>	Rodrigues et al. (2019)
<i>Pasteurella multocida, Peptococcus sp</i>	<i>Bergeyella, Capnocytophaga canimorsus, Pseudomonas</i>	Krumbeck et al. (2021)
<i>Filifactor villosus, Treponema sp., Lachnospiraceae bacillus, Peptostrep tococaceae, Porphyromonas Bacteroides</i>	<i>Moraxella sp., Bergeyella zoohelcum strain, Fusobacterium, Chlorobi bacterium, Porphyromonas circumdentaria</i>	Harris et al. (2015)
<i>TM7-3, Streptococcus, Anaerorhabdus, Actinomyces, Granulicatella, Petrimonas</i>	<i>Lysobacter, Suttonella, Bordetella, Clostridium</i>	Chaiyasut et al. (2022)
<i>Tannerella forsythia, Porphyromonas gingivalis</i>	-	

Microorganisms affecting Periodontal Disease

The concept of dysbiosis in periodontal disease has gained attention in recent years. It proposes that the development of periodontal disease is associated with changes in the composition and abundance of low-abundance bacteria within the periodontal pocket. An increase in the abundance of these bacteria and alterations in the proportion of microbial composition can lead to inflammation and subsequent destruction of the alveolar bone (Hajishengallis, 2014).

The immune response triggered by the oral microbiota plays a crucial role in maintaining tissue homeostasis and preventing damage to periodontal tissues (Dennison and Van Dyke, 1997). In a healthy state, the immune system effectively regulates the microbial community within the oral cavity, maintaining a balance between oral health and disease. However, when dysbiosis occurs, with an imbalance in the microbial community, the immune response may become dysregulated, leading to an inflammatory state that contributes to tissue destruction in periodontal disease.

The dysbiotic microbiota present in periodontal disease can elicit an immune response characterized by the release of pro-inflammatory mediators, such as cytokines and chemokines, by immune cells in the gingival tissues. These inflammatory mediators can recruit immune cells to the site of infection and activate destructive pathways, ultimately leading to the breakdown of periodontal tissues, including the alveolar bone.

Overall, the dysbiosis hypothesis suggests that changes in the composition and abundance of the oral microbiota can disrupt the immune homeostasis in the periodontal tissues, leading to an inflammatory response and subsequent destruction of the supporting structures of the teeth.

Filifactor alocis: Recent studies utilizing molecular biology techniques have identified *Filifactor alocis* (*F. alocis*), a gram-positive anaerobic bacterium, in diseased areas of periodontitis. The abundance and detection rate of *F. alocis* is higher in periodontitis compared to healthy sites, suggesting its potential as an indicator of periodontitis (Kumar *et al.*, 2005).

F. alocis has been found to invade the gingival epithelium and produce trypsin-like proteases that can withstand oxidative stress (Moffatt *et al.*, 2011). It also forms biofilms, allowing it to preferentially colonize the top of the gingival pocket near the soft tissue (Schlafer *et al.*, 2010).

F. alocis has been implicated in inducing apoptosis of gingival epithelial cells (GECs) and stimulating the production of inflammatory cytokines such as IL-1 β , IL-6, and TNF- α (Moffatt *et al.*, 2011). These findings suggest that *F. alocis* may be a significant pathogen involved in the formation of subgingival plaque biofilms and the development of periodontitis.

Therefore, the presence and quantity of *F. alocis* in the oral cavity have the potential to serve as biological markers for the diagnosis of periodontitis. However, further validation through larger clinical trials is necessary to confirm its clinical diagnostic utility.

Porphyromonas gingivalis: *Porphyromonas gingivalis* (*P. gingivalis*) is a gram-negative anaerobic bacterium that plays a significant role as a key pathogen in periodontal disease. It maintains the inflammatory flora associated with periodontal disease (Hajishengallis *et al.*, 2013). The abundance of *P. gingivalis* significantly increases in the oral cavity of patients with periodontal disease, particularly in those with refractory chronic periodontitis.

P. gingivalis has the ability to invade phagocytes and evade their killing mechanisms. The FimA component (pili) of *P. gingivalis* binds to CD14-TLR2/TLR1 receptors and triggers the entry of *P. gingivalis* into phagocytes. The CR3 receptor, which is involved in the uptake of apoptotic cells, also participates in the entry of *P. gingivalis* into cells (Sendide *et al.*, 2005).

The lipopolysaccharide (LPS) of *P. gingivalis* can activate Toll-like receptor 2 (TLR2) and TLR4, depending on the cell type (Kocgozlu *et al.*, 2009). *P. gingivalis* also secretes exotoxins, including various proteins such as enzymes and metabolic byproducts, which can harm the host when released. The release of cytokines, such as TNF- γ and TNF- α , from microglia is an outcome of appropriate concentrations of bacteria or their immunogenic components activating innate immune signaling pathways via TLR-2 and TLR-4 mechanisms. Prolonged release of cytokines can even lead to alterations in the permeability of the blood-brain barrier, allowing amyloid- β from the central nervous system to enter the systemic circulation (Weller *et al.*, 2009).

At appropriate concentrations of *P. gingivalis* LPS or peptidoglycan, the TLR-2 and TLR-4 signaling pathways are activated, along with the release of reactive oxygen species/nitrogen species (such as superoxide ions, iNOS/NO), cytokine secretion, and bacterial activation of the complement system. These processes collectively contribute to neuronal damage, chronic inflammation, and disease development (Qin *et al.*, 2005).

In summary, *P. gingivalis* is a significant periodontal pathogen that can invade host cells, activate immune response pathways, and release harmful exotoxins, leading to chronic inflammation and tissue destruction in periodontal disease.

Tannerella forsythia: *Tannerella forsythia* (*T. forsythia*) is a bacterial pathogen commonly associated with periodontal disease. It has several mechanisms that contribute to its virulence and survival in the oral environment.

T. forsythia can acquire MurNAc and peptidoglycan fragments (muropeptides) released by other bacteria during cell wall rupture associated with cell division in the subgingival environment. Additionally, *T. forsythia* has the ability to utilize host sialic acid (Neu5Ac) instead of MurNAc or peptide as a nutrient source during biofilm growth. This suggests that the bacteria can redirect sialic acid to metabolic pathways involved in peptidoglycan synthesis (Mayer *et al.*, 2019).

The peptidoglycan (PGN) of *T. forsythia* is typical of Gram-negative bacteria, consisting of an alternating arrangement of β -1,4 glycosidic bonds and multiple

glycan chains cross-linked by short peptides linked to MurNAc. This flexible network allows the bacterial cells to withstand intracellular osmotic pressure, preventing cell lysis (Walter and Mayer, 2019; Vollmer and Seligman, 2010).

T. forsythia expresses various virulence factors that are believed to contribute to its pathogenicity. The genome of *T. forsythia* contains numerous glycosidases and glycoactive enzymes, which may play a role in host-microbe interactions (Megson *et al.*, 2015). One notable virulence factor is the protease PrtH, which has been extensively studied. Additionally, a group of six proteases known as KLIKK proteases, with protein-like structures, has garnered attention for their potential role in virulence (Ksiazek *et al.*, 2015). The precise functions of these and other suggested virulence factors are still being investigated.

Overall, *T. forsythia* possesses mechanisms such as nutrient acquisition, cell wall structure, and expression of virulence factors that contribute to its survival and pathogenicity in the oral environment, particularly in the development and progression of periodontal disease.

Synergistic Effect Between Bacterial Communities

Periodontal disease is the result of a variety of microbial infections. Currently, it is believed that the synergistic effect of some microorganisms in the periodontal microbiota is the cause of the development of periodontal inflammation; these two or more bacteria provide substances conducive to reproduction, adhesion, invasion, and other functions. Interestingly, with the increase in bacterial numbers and clinical periodontal inflammation, the ecological succession from health to disease was mainly reflected in the emergence of alternating dominant microbiome members rather than the new microbiome species (Abusleme *et al.*, 2013).

***P. gingivalis* and *F. alocis*:** *P. gingivalis* and *F. alocis* are two bacterial species commonly associated with periodontal disease, and they have been found to interact and exhibit synergistic effects in promoting disease progression.

While *P. gingivalis* is widely studied and recognized as a key pathogen in periodontitis, it is generally believed that *P. gingivalis* alone may not be sufficient to cause infected gingivitis. It is a part of the normal oral flora and can act opportunistically during inflammation, exacerbating the disease process. *P. gingivalis* can also create a favorable microenvironment for other periodontal pathogens and aid in their manipulation of host immunity (Hajishengallis, 2014).

Studies have shown that *F. alocis* can have synergistic effects with other periodontal pathogens, including *P. gingivalis*. When *F. alocis* and *P. gingivalis* are co-cultured, they can promote the formation of biofilms and enhance the ability of *P. gingivalis* to invade epithelial cells through vesicle-mediated endocytosis (Aruni *et al.*, 2011). In vitro, community development experiments have indicated that *F. alocis* can be enriched in regions rich in another bacterium called *Fusobacterium nucleatum*, and they

synergistically promote each other's growth (Wang *et al.*, 2013).

Furthermore, in gingival tissues of patients with periodontitis, colocalized active aggregates of *P. gingivalis* and *F. alocis* have been observed in gingival microvessels. Both bacteria can invade the microvessels in the upper layer of tissue (lamina propria) and gingival epithelium, suggesting their cooperation in establishing chronic infection sites beneath the gingival epithelium and within the periodontal tissue. This synergistic environment may facilitate their spread to the gingival vascular plexus (Lee *et al.*, 2020).

These findings suggest that *F. alocis* and *P. gingivalis* can interact and cooperate to promote the development and progression of periodontal disease. The combined effects of these bacteria may contribute to the chronic infection and inflammation observed in periodontitis.

***P. gingivalis* and *T. forsythia*:** *P. gingivalis* and *T. forsythia* have been reported to exhibit synergistic effects in the context of periodontal disease. In vitro experiments have shown that *P. gingivalis* can enhance the ability of *T. forsythia* to attach to and invade epithelial cells (Inagaki *et al.*, 2006). When co-cultured, *P. gingivalis* and *T. forsythia* have been found to induce stronger mutagenesis effects compared to their individual cultures in a mouse model (Yoneda *et al.*, 2001).

Both *P. gingivalis* and *T. forsythia* utilize the type IX secretion system (T9SS) to secrete various virulence factors through the outer membrane (OM), which is considered a critical determinant of periodontal disease pathogenicity (Tomek *et al.*, 2014). These bacteria employ the T9SS to direct specific proteins, particularly those with a carboxy-terminal domain (CTD), to the outer membrane, where they can be expressed on the cell surface or secreted into the external environment (de Diego *et al.*, 2016). This process has been implicated in altering host immune responses to *P. gingivalis* and *T. forsythia* (Braun *et al.*, 2022).

Studies have also demonstrated that the combined infection of *T. forsythia* and *P. gingivalis* elicits a stronger specific systemic immune response, as evidenced by increased production of IgG antibodies (Zhu *et al.*, 2013). Furthermore, the co-infection of these two pathogens has been shown to induce characteristic periodontal inflammation and stimulation, leading to enhanced alveolar bone resorption in rat models. *P. gingivalis* and *T. forsythia* are routinely identified as part of the pathogenic biofilm found in deep periodontal pockets of patients with chronic periodontitis.

Overall, the interactions between *P. gingivalis* and *T. forsythia*, including their ability to enhance each other's pathogenicity, promote biofilm formation, and modulate host immune responses, contribute to the development and progression of periodontal disease.

***Fusobacterium nucleatum* and *P. gingivalis*:** In the subgingival biofilm, where various microbial components coexist, there is a stable environment and close contact that promotes the transfer of nutrients between the different species. One example is the close

relationship often observed between *P. gingivalis* and *Fusobacterium nucleatum* (*F. nucleatum*) in spatial imaging experiments of plaque biofilms (Mark Welch et al., 2016).

Both *F. nucleatum* and *P. gingivalis* produce lipopolysaccharides (LPS) that can act as stimulants to increase the number of neutrophils expressing receptor activator of nuclear factor kappa-B ligand (RANKL), which can affect the progression of periodontitis (Kim et al., 2021). Co-infection of *F. nucleatum* and *P. gingivalis* promotes their adherence to and invasion of gingival tissue cells (Li et al., 2015; Saito et al., 2012). Recent studies have shown that while *P. gingivalis* inhibits the invasion of oral epithelial cells by *F. nucleatum*, *F. nucleatum* actually promotes the invasion of oral epithelial cells by *P. gingivalis* (Zhang et al., 2022). *F. nucleatum* exerts its virulence through the paracellular pathway and collaborates with *P. gingivalis* to create a local environment that facilitates infection deep into the tissues (Jung et al., 2017).

Additionally, *F. nucleatum* creates a carbonophilic environment that is conducive to the growth of *P. gingivalis* and the metabolism of molecular oxygen and hydrogen peroxide (Diaz et al., 2002). This environment helps protect acid-sensitive bacteria from acidic conditions, providing a niche for their survival (Takahashi, 2003).

Overall, the interactions between *F. nucleatum* and *P. gingivalis* involve adherence, invasion of host cells, modulation of immune responses, and the creation of a microenvironment that promotes the survival and pathogenicity of these bacteria. These interactions contribute to the development and progression of periodontal disease.

Summary

This review provides a systematic summary of studies on dental plaque in cat gingivitis or periodontitis, including the analysis of major bacterial flora and comparisons with healthy oral sites. It also discusses the influence of certain bacteria on cell structure and the pathological mechanisms of periodontal disease. However, there are limitations to the current evidence and areas that require further research.

Firstly, the feline oral microbiome is a complex system, and existing studies may not have fully explored the disruption of bacterial balance or definitively established the etiology of periodontal disease in cats. While the review discusses the major bacterial flora, it may not have captured the full complexity and interactions within the oral microbiome.

Secondly, when investigating the synergistic relationships between different pathogenic bacteria, the studies reviewed may have only focused on the interactions between two selected pathogens in vitro. This approach may not fully reflect the co-functional dynamics at the disease site in vivo.

Therefore, future studies on the feline oral microbiome should consider investigating the interactions within the microbiome as well as between pathogens and host immunity. This broader approach can provide a more comprehensive understanding of the mechanisms underlying periodontal disease in cats and potentially contribute to the development of novel treatments.

Table 2 Influence of Mouth Spray or other factors on the oral microbiome composition in cats

Influence factor	Influence time	Oral microbiome composition in cats	Reference
Gallic Acid-Containing Mouth Spray	42 days	Mouth spray reduced the salivary microbial load frequency of phyla Firmicutes, TM7, Actinobacteria, OD1, Fusobacteria, Chloroflexi, WS6, GN02, Spirochaetes, and Planctomycetes. The phylum Verrucomicrobia was completely eradicated by mouth spray.	Chaiyasut et al. (2024)
Dry diet	-	The most enriched bacteria in cats eating dry diets exclusively included OTUs from the following genera: Actinobacillus, Achole plasma, Treponema, and Porphyro monas.	Adler et al. (2016)
Wet diet	-	The remaining 35 % of OTUs were significantly more abundant in dental plaque from cats consuming a wet diet. These bacteria were primarily Proteobacteria from the Neisseriaceae family (38 %), including Conchiformibius kuhniae.	Adler et al. (2016)
34 free-roaming cats	5 months	Twenty aerobic bacterial genera were isolated; ten of them were the predominant genus in at least one cat , 58.8% were Gram-positive, and 41.2% were Gram-negative. Alpha-haemolytic Streptococcus (23.5%), Staphylococcus (17.6%), Neisseria (15.7%), and Pasteurella (11.8%) were the most frequent predominant genera (percentage of isolates as the predominant genus over 34 individuals).	Whyte et al. (2017)

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