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Molecular detection of feline and canine periodontal pathogens

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ABSTRACT

Periodontal disease is the most common infectious disease of cats and dogs which are strongly associated with periodontal pathogens. The primary etiologic factor in the formation of periodontal disease is microbial dental plaque accumulation on teeth. In our research, we aimed to investigate the presence of periodontal disease-related bacterial species in dental plaques of cats and dogs. Specimens collected from 50 cats and 51 dogs with periodontal disease examined in terms of periodontal pathogens by polymerase chain reaction (PCR) using primers directed to 16S rRNA and tdpA genes. Our findings indicate the presence of periodontal disease-related pathogens, especially *Porphyromonas gingivalis* (cats 96%, dogs 88%), *Prevotella nigrescens* (cats 90%, dogs 57%) and, *Porphyromonas gulae* (cats 70%, dogs 39%). In addition, the prevalence of *Tannerella forthysia* (cats 2%, dogs 4%) well-known pathogen in cats and dogs were isolated with an extremely low percentage.

Furthermore, our results suggest that the feline oral cavity microbiota has considerably more diversity than dogs. Consequently, daily oral hygiene practices may become essential for controlling the pathogenic bacteria which have clinical importance and in preventing the propagation of microorganisms in the oral cavity of cats and dogs.

1. Introduction

The microbial population colonizing on the teeth begins dental infections such as periodontal diseases, gingivitis, and pulpitis in humans, cats and dogs (Hale, 2009; Munemasa et al., 2000). Periodontal disease is a set of inflammatory conditions affecting the tissues surrounding the teeth. Feline and canine specifications as age, species, breed, genetics, diet, health status, habitat, the frequency of dental care and bacterial flora condition of the oral cavity may have a role in the development of diseases (Kim & Amar, 2006; Niemiec, 2012). The disease is common in cats and dogs with a prevalence of 70% and 80%, respectively (Booij-Vrieling et al., 2010). While Gram-positive bacteria species are predominant in healthy dogs, Gram-negative anaerobes prevail in supragingival and subgingival plaques in dogs in the course of periodontal diseases (Ebrahimi, O. & Khoshnevisan, 2010; Forsblom et al., 2002; Harvey, Thornsberry & Miller, 1995). Both Gram-positive and Gram-negative bacteria may lead to inflammation and the gingival destruction of periodontal tissue as well as the loss of alveolar bone in humans and animals with periodontal disease. Also, anaerobe bacteria may cause releasing of enzymes and endotoxins during the formation of periapical lesions. *Porphyromonas* sp. and *Prevotella* sp. can be found in dental plaque and periodontal pockets. In particular, *P. gingivalis* contributes to chronic periodontal disease and inhibits the migration of PMNs that pass through the epithelial barrier (Dahlen, 2002; Forsblom et al., 2002). The *Porphyromonas* sp. species have also appropriate virulence factors that can cause periodontal disease and stimulate an appropriate humoral immune response (Adler, Malik & Gina, 2016).

Many studies indicated that diet consumption has an important effect on the formation of the oral microbiome and periodontal disease. Soften wet diets have been associated with the prevalence and severity of periodontal disease in cats and dogs. Therefore, it is recommended that feeding with dry food diet has a positive effect on oral health and reduces the formation of dental residues and periodontal disease (Adler et al., 2016; Gawor et al., 2006).

The participation of potential zoonotic and periodontopathic bacteria in the oral flora of cats and dogs may cause public health problems due to bite wound infections (Booij-Vrieling et al., 2010; Khazandi et al., 2014; Yamasaki et al., 2012). The infection rates are between 4–25% and 20–50% in the case of cats and dogs bite wounds, and the symptoms appear within 24 h. Furthermore, bites can also cause a systemic infection which results in 6.7% death annually (Griego, Rosen, Orengo & Wolf, 1995; Talan, Citron, Abrahamian, Moran & Goldstein, 1999). On average, up to 15–20% of dog bites and approximately 30–50% of cat bites have been infected (Brook, 2003; Centers for

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Disease Control and Preention, 2015; Rothe, Tsokos & Handrick, 2015).

The periodontal pathogens such as *C. sputigena, P. gingivalis, P. nigrescens, E. corrodens, C. rectus, C. ochracea, A. actinomycetemcomitans, T. forsythia*, and *T. denticola* have been reported to be isolated from saliva samples of humans (Piau, Arvieux, Bonnaure-Mallet & Jolivet-Gougeon, 2013; Tamura et al., 2006). Additively, these bacteria species from oral microbiome of dogs and cats can cause many diseases in humans such as *Aggregatibacter actinomycetemcomitans* (brain abscesses, endocarditis, rheumatoid arthritis-a potential trigger of the autoimmune disease) (Henderson, Wilson, Sharp & Ward, 2002; Konig et al., 2016), *Campylobacter rectus* (periodontal disease-because of increased salivary estradiol concentrations during pregnancy) (Mahlen & Clarridge, 2009), *Capnocytophaga ochracea* (intrauterine infections, endocarditis), *Capnocytophaga sputigena* (iliopsoas abscess) (Desai, Harrison & Murphy, 2007), *Eikenella corrodens* (sinusitis, arthritis, endocarditis, pancreatic abscesses, vertebral osteomyelitis) (Paul & Patel, 2001), *Porphyromonas gingivalis* (rheumatoid arthritis, bacterial vaginosis, osteomyelitis) (Gaetti-Jardim, Marqueti, Faverani & Gaetti-Jardim, 2010; Venkataraman & Almas, 2015; Wegner et al., 2010), *Porphyromonas gulae* (periodontal disease; bind to human oral epithelial cells) (Hamada et al., 2008; Yamasaki et al., 2012), *Prevotella intermedia* (gingivitis-during pregnancy, cystic fibrosis) (Borgo, Rodrigues, Feitosa, Xavier & Avila-Campos, 2014; Gilpin et al., 2017), *Prevotella nigrescens* (carotid atherosclerosis) (Yakob et al., 2011), *Tannerella forsythia* (atherosclerosis, osteomyelitis) (Ardila, Perez-Valencia & Rendon-Osorio, 2015; Gaetti-Jardim et al., 2010), *Treponema denticola* (bacterial vaginosis, bone infections) (Africa, Nel & Stemmet, 2014). Therefore, a meticulous oral hygiene application on pet animals is of paramount importance.

The aim of this study was to determine the distribution of periodontal pathogens (*A. actinomycetemcomitans, C. rectus, C. ochracea, C. sputigena, E. corrodens, P. gingivalis, P. gulae, and P. intermedia*) in cats and dogs dental plaque samples by using primers directed to 16S rRNA and tdpA genes with PCR.

2. Materials and methods

2.1. Collection of samples

In this study, samples were collected from veterinary clinics in İzmir province and districts between November 2017 and March 2018. Dental plaque swab samples were obtained and transported in Stuart agar from the maxillary molar region of 51 dogs and 50 cats with periodontal disease. Meanwhile, specimens were transferred into plastic falcon tubes and crushed in 5 ml of sterile distilled water. Then, they were brought to Aydın Adnan Menderes University Veterinary Faculty Microbiology Department under the cold chain.

2.1.1. DNA extraction of bacteria

Centrifugation was applied to the samples at 10,000 xg for 5 min and, remaining sediment gathered at the bottom of the tube was dissolved in 100 µl sterile saline. DNA was extracted from bacteria colonies of *Porphyromonas gingivalis* (ATCC 33,277), Treponema denticola (ATCC 35,405), Tannerella forsythia (ATCC 43,037), Capnocytophaga ochracea (ATCC 27,872), Capnocytophaga sputigena (ATCC33612) Prevotella intermedia (ATCC 25,611), *Prevotella nigrescens* (ATCC 33,563), Campylobacter rectus (ATCC 33,238), Aggregatibacter actinomycetemcomitans (ATCC 33,384), Eikenella corrodens (ATCC 23,834) and, *Porphyromonas gulae* (ATCC 51,700) and used for positive control. Subsequently, the bacterial DNA extraction process was carried out for each sample by using a DNA extraction kit (Thermo Fisher®) as indicated by the manufacturer.

2.1.2. Design of primer

PCR assay conducted by using species-specific primers after the extraction (Table 1).

The universal primer sets designed for use as a positive control for the detection of bacteria (Doungudomdacha, Rawlinson & Douglas, 2000), P. gingivalis, T. denticola, T. forsythia, C. ochracea, C. sputigena, P. intermedia, P. nigrescens, A. actinomycetemcomitans, C. rectus, and E. corrodens from cats and dogs oral cavity (Ashimoto, Chen, Bakker & Slots, 1996; Conrads et al., 1996; Kuboniwa et al., 2004; Watanabe & Frommel, 1996). Moreover, it was planned to identify of P. gulae which can be isolated from gingival cavities in cats and dogs, excluded from human originated P. gingivalis strains in the study (Hamada et al., 2008; Kato et al., 2011).

2.1.3. Polymerase chain reaction (PCR) stage

5 µl DNA sample and 45 µl PCR master mixes were used in the amplification of the universal primer sets to the detection of total bacteria. Thereafter, the amplification was applied under the conditions was pre-denaturation at 95 °C for 5 min, 1 min denaturation at 95 °C, 1 min annealing at 55 °C, 1 min elongation at 72 °C with 30 cycles and a final elongation at 72 °C for 10 min with 1 cycle (Doungudomdacha et al., 2000). DNAs of the samples identified as positive for evaluation of the presence of total bacteria as a result of amplification by universal primers. Then, all positive samples practiced in multiplex PCR, including 5 µl of DNA sample and 45 µl of PCR master mix. Afterwards, amplification was carried out under the following conditions as 95 °C for 5 min for initial denaturation, 94 °C for 30 s, 62 °C for 30 s, 72 °C with 30 cycles for 30 s, 72 °C for 5 min at a final elongation 1 cycle. The PCR products were soon after electrophoresed at 80 V/cm power for 40 min with a 2% agarose gel which containing ethidium bromide. At the end of the electrophoresis, the gel screened via Vilber Lourmat UV transilluminator system and band size was searched at the base ranges of target size (Table 1) (Hamada et al., 2008; Kato et al., 2011).

3. Results

Analysis of all dental plaque swab samples collected from cats and dogs by using primers directed to 16S rRNA and tdpA genes with PCR yielded a great number of positive results in this study. The isolates obtained from cats (48/50, 45/50) and dogs (45/51, 29/51) swap samples were identified as *Porphyromonas gingivalis* and *Prevotella nigrescens*, respectively. In the present study, Capnocytophaga ochracea and Capnocytophaga sputigena were also detected from only 4 cats. Besides, *Porphyromonas gingivalis* was detected in almost all cats and dogs. Forty-eight of 50 cats (96%) and forty-five of 51 dogs (88, 23%) were shown possess to that species (Fig 1).

In contrast, the detection rates of 3 species (T.forsythia, C. ochracea, and C. sputigena) in cats and of 5 species (T. forsythia, C. ochracea, C. sputigena T. denticola and, E. corrodens) in dogs showed that the prevalence was lower than 10%. E. corrodens in cats and, P. intermedia, A. actinomycetemcomitans, and C. rectus in dogs were also isolated from the swab samples with less than 30% percentage. Remarkably, C. sputigena and C. ochracea species that were not detected in dogs swab specimens (0%), although it was detected in 2% of cats even if with low percentages (Fig. 2).

P. gulae, P. gingivalis, and P. nigrescens were also the most frequently detected species in dogs. The detection percentages of these bacteria were 39, 2%, 88, 2% and 56, 8%, respectively (Fig 3).

4. Discussion

Periodontal disease is one of the most common infectious disorders in cats and dogs (Niemiec, 2012). Gram-negative bacteria such as *A. actinomycetemcomitans, T. forsythia, Campylobacter* spp., *Capnocytophoga* spp., *E. corrodens, P. gingivalis, P. intermedia*, and *T. denticola* can contribute to forming of subgingival plaque and particularly have importance in bite wounds (He and Shi, 2009). Some studies have shown that the cat's oral cavity is shifted towards anaerobic gram-negative

Table 1
PCR primer sets used for the detection of the bacterial species.

Target species	Sequences (5′-3′)	Target gene	Size (bp)	References
Universal primer (positive control)	AGA GTT TGA TCM TGG CTC AG CTG CTG CSY CCC GTA G	16S rRNA	315	Doungudomdacha et al., 2000
Porphyromonas gingivalis	CCG CAT ACA CTT GTA TTA TTG CAT GAT ATT AAG AAG TTT ACA ATC CTT AGG ACT GTC T	16S rRNA	267	Kato et al., 2011.
Treponema denticola	AAG GCG GTA GAG CCG CTC A AGC CGC TGT CGA AAA GCC CA	tdpA	311	Watanabe and Frommel, 1996
Tannerella forsythia	GCG TAT GTA ACC TGC CCG CA TGC TTC AGT GTC AGT TAT ACC T	16S rRNA	641	Ashimoto et al., 1996
Capnocytophaga ochracea	AGA GTT TGA TCC TGG CTC AG GAT GCC GTC CCT ATA TAC TAT GGG G	16S rRNA	185	Conrads et al., 1996
Capnocytophaga sputigena	AGA GTT TGA TCC TGG CTC AG GAT GCC GCT CCT ATA TAC CAT TAG G	16S rRNA	185	Conrads et al., 1996
Prevotella intermedia	TTT GTT GGG GAG TAA AGC GGG TCA ACA TCT CTG TAT CCT GCG T	16S rRNA	575	Ashimoto et al., 1996
Prevotella nigrescens	ATG AAA CAA AGG TTT TCC GGT AAG CCC ACG TCT CTG TGG GCT GCG A	16S rRNA	804	Ashimoto et al., 1996
Campylobacter rectus	TTT CGG AGC GTA AAC TCC TTT TC TTT CTG CAA GCA GAC ACT CTT	16S rRNA	598	Ashimoto et al., 1996
Aggregatibacter actinomycetemcomitans	CTA GGT ATT GCG AAA CAA TTT G CCT GAA ATT AAG CTG GTA ATC	16S rRNA	262	Kuboniwa et al., 2004
Eikenella corrodens	CTA ATA CCG CAT ACG TCC TAA G CTA CTA AGC AAT CAA GTT GCC C	16S rRNA	688	Ashimoto et al., 1996
Porphyromonas gulae	TTG CTT GGT TGC ATG ATC GG GCT TAT TCT TAC GGT ACA TTC ACA	16S rRNA	314	Doungudomdacha et al., 2000

rods with higher gingival index scores (Adler et al., 2016). The most prevalent species were detected as *A. actinomycetemcomitans* (64%), *P. gulae* (70%), *P. gingivalis* (96%), *P. intermedia* (60%), *P. nigrescens* (90%) in cats and *P. gulae* (39%), *P. gingivalis* (88%) and *Prevotella nigrescens* (57%) in dogs in our study, respectively. It has been reported that the combinations of *A. actinomycetemcomitans* and *P. gingivalis* contributed to the formation of deepened pockets in periodontal disease (Samaranayake, 2012). We observed the prevalence of *Prevotella intermedia* (60%; 20%), *A. actinomycetemcomitans* (64%; 24%), *Porphyromonas gulae* (70%; 39%), *Prevotella nigrescens* (90%; 57%) in cats much higher than dogs, respectively. In addition to these results, *P. nigrescens* (57%) interestingly found highly prevalent.

The acquired data from this study confirmed that *C. ochracea* and *C. sputigena* species were not encountered in dogs but found in cats with a low rate (4%) which this bacterium can be associated with periodontal disease in cats. Although *T. denticola* and *E. corrodens* were identified with 6% and 4% from all dogs plaque samples, it could be regarded as determining. The prevalence of *P. gulae, P. nigrescens*, and *P. gingivalis* were detected highly in cats plaque samples (70%, 90%, 96%) and dogs plaque samples (39%, 57%, 88%) by using PCR. Findings of *P. gingivalis* from dental plaque samples were noticeably high in cats and dogs. Therefore, *P. gingivalis* can be evaluated an opportunistic pathogen

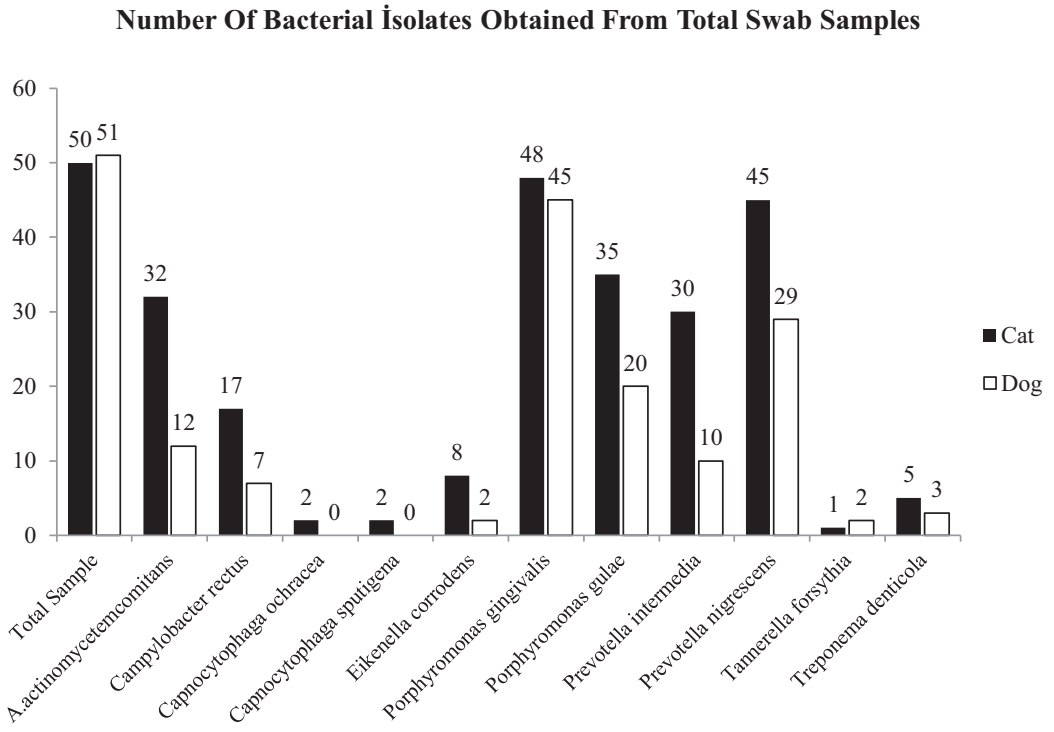

Fig. 1. Distribution of isolated periodontal bacteria in cats and dogs.

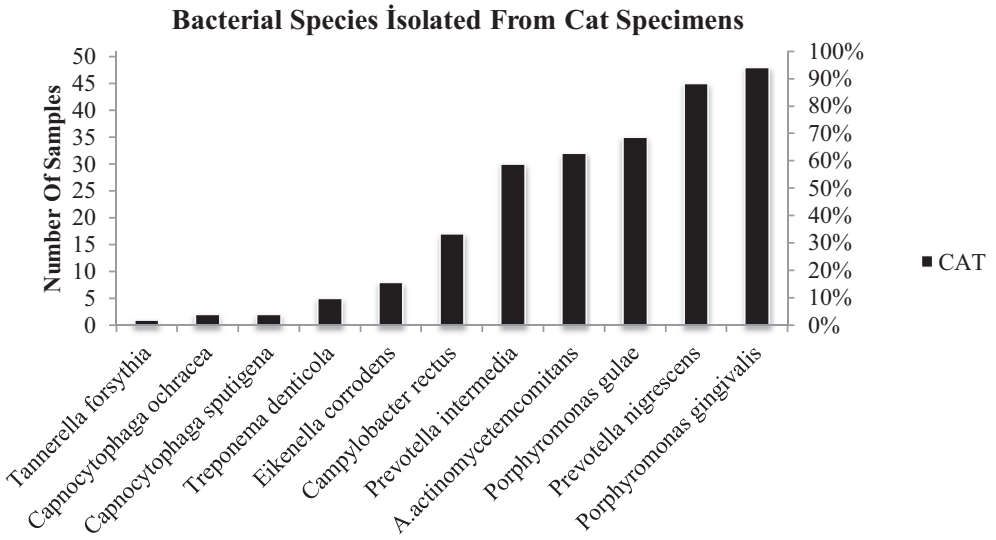

Fig. 2. Bacterial isolates identified from cats by PCR.

which initiates the infection (Fujise, Hamachi, Inoue, Miura & Maeda, 2002; van Winkelhoff, Loos, van der Reijden & van der Velden, 2002).

T. forsythia has also been implicated as significant periodontopathogens and conceivably can be found 90% with varying stages in periodontal disease in Booij-Vrieling et al. (2010), Perez-Salcedo, Herrera and Esteban-Saltiveri (2013), Zarco, Vess and Ginsburg (2012). In a recent study, we were isolated 2% in cats dental plaque samples. These discrepancies can common likely be attributed to the fact that cats are contingently at different stages of periodontal disease.

P. gulae reported as the most common type in dogs (Forsblom et al., 2002; Hale, 2009). However, in our study, we were rarely (39%) identified this bacterium from dogs dental samples. *C. rectus* (67%) has been described as the most common genera isolated from dental plaque specimens collected from dogs (Yamasaki et al., 2012). However, we were also rarely detected *C. rectus* (14%) in our samples. *P. gingivalis, P. intermedia*, and *P. nigrescens* are known as Black-pigmented anaerobes (BPA) have been associated as common pathogens with the periodontal disease in both genera. Especially, *P. gingivalis* and *P. nigrescens* showed a correlation in 90% of the cases.

Some studies showed that *Tannerella* sp. and *Porphyromonas* sp. were the most common oral flora bacteria isolated from cats. However, *Porphyromonas* sp. found to be the dominant species in cats besides the low percentage of *Tannerella* sp. (2%) (Kasempimolporn, Benjavongkulchai, Saengseesom & Sitprija, 2003). In addition, *P. gulae* has been evaluated as one of the most dominant pathogen in the oral cavity (Allaker, Langlois & Hardie, 1994; Kato et al., 2011). The 38–76% of anaerobic bacteria, for instance, *Prevotella* sp., *Porphyromonas* sp., has been reported to be present bite wounds of cats and dogs. The isolation of *P. gulae, P. nigrescens*, and *P. gingivalis* from dental plaque samples supports our findings (Arakawa et al., 2000; Aydin, 2004; Foschi et al., 2005; Munemasa et al., 2000). Moreover, Senhorinho et al. (2011) reported that 92% of *P. gulae* has been isolated in their study. However, we were interestingly detected in 39% in our study. We also demonstrate that the results obtained from PCR support the presence of *P. gulae, P. nigrescens* and *P. gingivalis* in cats which are significantly associated with periodontal disease.

Although the genetic literature for periodontal disease is more important than caries, micronutrient deficiencies such as vitamin C, vitamin D or vitamin B12 may be associated with the onset and progressive in periodontal disease in cats and dogs. Furthermore, genes involved in enamel formation in humans (AMELX, AMBN, ENAM,

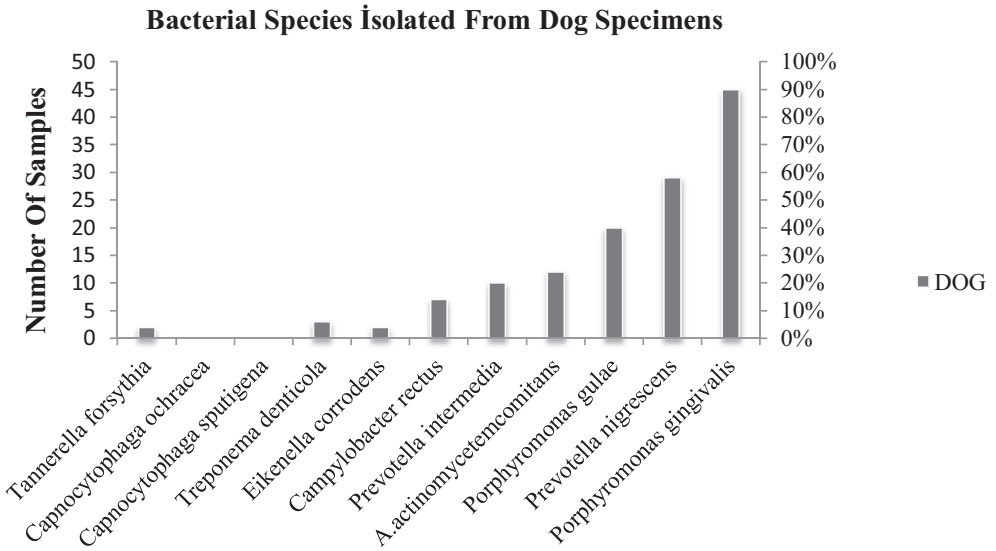

Fig. 3. Bacterial isolates identified from dogs by PCR.

TUFT, MMP20, and KLK4), saliva characteristics (AQP5), have the greatest effect on caries (Chapple, Bouchard, Cagetti, Campus & Carra, 2017). Therefore, it would be beneficial to investigate these formation features in cats and dogs presenting with periodontal disease.

5. Conclusions

Our results suggest that the feline oral cavity considerably has more diversity of microbiota than dogs. Bacteria such as P. gulae, P. nigrescens, and P. gingivalis were the major species in dental plaque samples collected from cats and dogs. Similarly, P. gingivalis and P. nigrescens known to be important pathogens for periodontitis in humans and they were highly identified in this study. Thus, the periodontal pathogens detected in cats and dogs should be eliminated by improving oral hygiene.

In addition to oral health control, high protein-based nutrient consumption promotes bacterial composition in oral flora and the oral cavity. Besides, feeding with a well-formulated dry food diet may be a positive effect on oral health and reduces the formation of dental residues and periodontal disease. Bacterial synergism in conjunction with virulence factors of periodontal diseases and the effects of nutrition on the development of the oral microbiome in pet animals should be investigated.

Finally, the results of the study can also provide measures by which veterinary doctors specialized in dentistry can monitor the risk of developing periodontal infections from cats and dogs oral pathogens through both diet control and assessment of plaque and calculus.

Declaration of Competing Interest

There is not any commercial firm played role in the study design nor in the collection, analysis and interpretation of data, nor in the decision to submit the manuscript for publication. None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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