Molecular detection of feline and canine periodontal pathogens

Volkan Özavci a, Göksel Erbas b, Uğur Parin b, Hafize Tuğba Yüksel b, Şükrü Kirkan b

a Department of Microbiology, Faculty of Veterinary Medicine, Yozgat Bozok University, Yozgat, Turkey
b Department of Microbiology, Faculty of Veterinary Medicine, Aydın Adnan Menderes University, Aydın, Turkey

ARTICLE INFO

Keywords:
Periodontal disease
Cat
Dog
PCR

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 forsythia (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 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, Oreno & Wolf, 1995; Talan, Citron, Abrahimian, 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...
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. spitygina, 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 mixtures 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 tcpA 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) samples were identified as Porphyromonas gingivalis and Prevotella nigrescens, respectively. In the present study, Capnocytophaga ochracea and Capnocytophaga spitiygena 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. spitiygena) in cats and of 5 species (T. forsythia, C. ochracea, C. spitiygena 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. spitiygena 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 (Table 1).

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., Capnocytophaga 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
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%; 24%), *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. spuitigena* 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.

**Table 1**

| Target species (positive control) | Sequences (5′-3′) | Target gene | Size (bp) | References |
|----------------------------------|------------------|-------------|-----------|------------|
| Universal primer (positive control) | AGA GTT TGA TCM TGG CTC AG | 16S rRNA | 315 | Doungudomdacha et al., 2000 |
| *Porphyromonas gingivalis* | CCG CAT ACA CTG TTA TTG CAT GAT ATT | 16S rRNA | 267 | Kato et al., 2011. |
| *Treponema denticola* | AAG GGG GTA GAG GCC TCT A | 16S rRNA | 311 | Watanabe and Frommel, 1996 |
| *Tannerella forsythia* | GGG TAT GTA ACC TGC CCG CA | 16S rRNA | 641 | Ashimoto et al., 1996 |
| *Capnocytophaga ochracea* | AGA GTT TCA TCC TGG TCT AG | 16S rRNA | 185 | Conrads et al., 1996 |
| *Capnocytophaga spuitigena* | AGA GTT TGA TCC TGG CTC AG | 16S rRNA | 185 | Conrads et al., 1996 |
| *Prevotella intermedia* | TTT GTT GGG GAG TAA AGC GGG | 16S rRNA | 575 | Ashimoto et al., 1996 |
| *Prevotella nigrescens* | ATG AAA CAA AGG TTT TCC GGT AAG | 16S rRNA | 804 | Ashimoto et al., 1996 |
| *Campylobacter rectus* | TTT CCG AGC GTA AAC TCC TTC TC | 16S rRNA | 598 | Ashimoto et al., 1996 |
| *Aggregatibacter actinomycetemcomitans* | CTA GGT ATT GGG AAA CAA TTT G | 16S rRNA | 262 | Kubonisiwa et al., 2004 |
| *Eikenella corrodens* | CTA ATA CGG CAT ACG TCC TAA G | 16S rRNA | 688 | Ashimoto et al., 1996 |
| *Porphyromonas gulae* | GTG CTT GTG TGC ATG ATC ACA TTA | 16S rRNA | 314 | Doungudomdacha et al., 2000 |

**Number Of Bacterial Isolates Obtained From Total Swab Samples**

| Target species | Cat | Dog |
|----------------|-----|-----|
| *Actinobacillus actinomycetemcomitans* | 50 | 51 |
| *Campylobacter rectus* | 32 | 17 |
| *Capnocytophaga ochracea* | 12 | 7 |
| *Capnocytophaga spuitigena* | 17 | 2 |
| *Eikenella corrodens* | 17 | 2 |
| *Porphyromonas gulae* | 48 | 45 |
| *Porphyromonas intermedia* | 35 | 45 |
| *Prevotella nigrescens* | 30 | 29 |
| *Treponema denticola* | 22 | 12 |

**Fig. 1.** Distribution of isolated periodontal bacteria in cats and dogs.
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-Salitteri (2013), Zarco, Vess and Ginsburg (2012). In a recent study, we were isolated 2% in cats dental plaque samples. These discrepancies can commonly 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 28–76% of anaerobic bacteria, for instance, *Prevotella*, *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,
TUFT, MMP20, and KLK4), saliva characteristics (AQP5), have the greatest effect on caries (Chapple, Bouchard, Catgut, Campus & Carr, 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 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 residue 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.

Acknowledgments

This research was funded by the Scientific Research Council of Yozgat Bozok University (Project No: 6602c ZF/16-40).

References

Adler, C. J., Malik, Richard, & Gina, V. (2016). Diet may influence the oral microbiome composition in cat. Microbiome, 4(1), 23.
Africa, C. W., Nel, J., & Stemmet, M. (2014). Anaerobes and bacterial vaginosis in pregnancy: Virulence factors contributing to vaginal colonization. International Journal of Environmental Research and Public Health, 11(7), 6979-7000.
Allaker, R. P., Langlois, T., & Hardie, J. M. (1994). Prevalence of eikenella corrodens and actinobacillus actinomycetemcomitans in the dental plaque of dogs. The Veterinary Record, 134(20), 519-520.
Arakawa, S., Nakajima, T., Ishikura, H., Ichinose, S., Ishikawa, I., & Tsuchida, N. (2000). Comparison of periodontal pathogens between cats and dogs potential bite wound pathogens. Journal of Microbiological Methods, 41, 207-220.
Foschi, F., Cervini, F., Montebuogni, L., Stashenko, P., Sambrì, V., & Prati, C. (2005). Detection of bacteria in endodontic samples by polymerase chain reaction assays and association with defined clinical signs in Italian patients. Oral Microbiology and Immunology, 20(5), 289-295.
Fujise, O., Hamachi, T., Inoue, K., Miura, M., & Maeda, K. (2002). Microbiological markers for prediction and assessment of treatment outcome following non-surgical periodontal therapy. Journal of Periodontology, 73(11), 1253-1259.
Griego, R. D., Rosen, T., Orengo, I. F., & Wolf, J. E. (1995). Dog, cat, and human bites: A review. Journal of Applied Oral Science: Revista FOB, 3(2), 47-58.
Harvey, C. E., Thornberry, C., & Miller, B. R. (1995). Subgingival bacteria composition of culture results in dogs and cats with gingivitis. Journal of Veterinary Dentistry, 12(1), 147-156.
Henderson, B., Wilson, M., Sharp, L., & Ward, J. M. (2002). Actinobacillus actinomycetemcomitans. Journal of Medical Microbiology, 51, 1013-1020.
Kato, Y., Shirai, M., Murakami, M., Mizusawa, T., Hagimoto, A., Wada, K., et al. (2011). Molecular and antigenic similarities of the fimbrial major components between porphyromonas gulae and P. gingivalis. Virillogia Microbiology, 128(1-2), 108-117.
Harvey, C. E., Thornberry, C., & Miller, B. R. (1995). Subgingival bacteria composition of culture results in dogs and cats with gingivitis. Journal of Veterinary Dentistry, 12(1), 147-156.
Kato, Y., Shiraiz, M., Murakami, M., Mizusawa, T., Hagimoto, A., Wada, K., et al. (2011). Molecular detection of human periodontal pathogens in oral swab specimens from dogs in Japan. Journal of Veterinary Dentistry, 28, 84-49.
Khadzid, M., Bird, P. S., Owens, J., Wilson, G., Meyer, J. N., & Trout, D. J. (2014). In vitro efficacy of cefovecin against anaerobic bacteria isolated from subgingival plaque of dogs and cats with periodontal disease. Anaerobe, 28, 104-108.
Kim, J., & Amar, S. (2006). Periodontal disease and systemic conditions: A bidirectional relationship. Oral Microbiology and Immunology, 21(4), 292-295.
Kubota, K., Amano, A., Kimura, K. R., Sekine, S., Kato, Y., Yamamoto, Y., et al. (2001). Quantitative detection of periodontal pathogens using real-time polymerase chain reaction with Taqman probes. Oral Microbiology and Immunology, 19, 168-176.
Mahnken, S. D., & Claridge, J. E., Jr (2009). Oral abscess causing canine (<i>Sebaldia</i>) in a dog. Journal of Clinical Microbiology, 47, 848-851.
Munemasa, T., Takemoto, T., Dahlen, G., Hino, T., Shiba, H., Ogawa, T., et al. (2000). Aggregatibacter actinomycetemcomitans induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis. Science Translational Medicine, 3(86RA176).
Nisizuka, I., Amano, K., Kimura, K. R., Sekine, S., Kato, Y., Yamamoto, Y., et al. (2004). Quantitative detection of periodontal pathogens using real-time polymerase chain reaction with Taqman probes. Oral Microbiology and Immunology, 19, 168-176.
Paul, K., & Patel, S. S. (2001). Eikenella corrodens infections in children and adolescents: Case reports and review of the literature. Clinical Infectious Diseases: An Official Publication of The Infectious Diseases Society of America, 33, 54–61.
Perez-Salcedo, L., Herrera, D., Esteban-Saldivier, D., Leon, R., Jesusle, I., Torre, C., et al.

Veterinary and Animal Science 8 (2019) 100069
(2013). Isolation and identification of *Porphyromonas* spp. and other putative pathogens from cats with periodontal disease. *Journal of Veterinary Dentistry, 30*, 208–213.

Piu, C., Arvieux, C., Bonnaure-Mallet, M., & Jolivet-Gougeon, A. (2013). *Capnocytophaga* spp. involvement in bone infections: A review. *International Journal of Antimicrobial Agents, 41*, 509–515.

Rothe, K., Tsokos, M., & Handrick, W. (2015). Animal and human bite wounds. *Deutsches Arzteblatt International, 112*, 433–443.

Samaranayake, L. (2012). Normal oral flora, the oral ecosystem and plaque biofilms. *Essential Microbiology for Dentistry, 392*, 291–294.

Talan, D. A., Citron, D. M., Abrahamian, F. M., Moran, G. J., & Goldstein, E. J. (1999). Bacteriologic analysis of infected dog and cat bites. Emergency medicine animal bite infection study group. *The New England Journal of Medicine, 340*, 85–92.

Tamura, K., Nakano, K., Hayashibara, T., Nomura, R., Fujita, K., Shintani, S., et al. (2006). Distribution of 10 periodontal bacteria in saliva samples from Japanese children and their mothers. *Archives of Oral Biology, 51*, 371–377.

van Winkelhoff, A. J., Loos, B. G., van der Reijden, W. A., & van der Velden, U. (2002). *Porphyromonas gingivalis, Bacteroides forsythus* and other putative periodontal pathogens in subjects with and without periodontal destruction. *Journal of Clinical Periodontology, 29*(11), 1023–1028.

Venkataraman, A., & Almas, K. (2015). Rheumatoid arthritis and periodontal disease. An update. *The New York State Dental Journal, 81*, 30–36.

Watanabe, K., & Frommel, T. O. (1996). *Porphyromonas gingivalis, actinobacillus actinomycetemcomitans* and *treponema denticola* detection in oral plaque samples using the polymerase chain reaction. *Journal of Clinical Periodontology, 23*, 212–219.

Wegner, N., Wait, R., Sroka, A., Eick, S., Nguyen, K. A., Lundberg, K., et al. (2010). Peptidylarginine deiminase from *porphyromonas gingivalis* citrullinates human fibrinogen and alpha-2-antiplasmin: Implications for autoimmunity in rheumatoid arthritis. *Arthritis and Rheumatism, 62*, 2662–2672.

Yakob, M., Soder, B., Meurman, J. H., Jogerstrand, T., Nowak, J., & Soder, P. O. (2011). Prevotella nigrescens and *porphyromonas gingivalis* are associated with signs of carotid atherosclerosis in subjects with and without periodontal disease. *Journal of Periodontal Research, 46*, 749–755.

Yamasaki, Y., Nomura, R., Nakano, K., Naka, S., Matsumoto-Nakano, M., Asai, F., et al. (2012). Distribution of periodontopathic bacterial species in dogs and their owners. *Archives of Oral Biology, 57*, 1183–1188.

Zarco, M. F., Vess, T. J., & Ginsburg, G. S. (2012). The oral microbiome in health and disease and the potential impact on personalized dental medicine. *Oral Diseases, 18*, 109–120.