ISSN: 18158129 E-ISSN: 18151027
Volume 21, Issue 02, February, 2025

Prevotella intermedia as a prognostic biomarker for periodontitis in dogs.

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ABSTRACT— Periodontal disease is one of the most common disorders in the oral cavity of dogs and humans. Periodontitis, the irreversible periodontal disease, arises progressively from gingivitis, the reversible inflammatory condition caused by dental plaque. Although the etiology of periodontitis has been widely studied in humans, it is still insufficient for the etiological studies on periodontitis in dogs. Many studies have reported that human periodontitis-related bacteria are putative pathogens responsible for periodontitis in dogs. However, most of these studies have focused on the appearance of a specific microbiome, and most of the cohort studies have insufficient sample sizes to generalize their results. In the present study, subgingival samples collected from 336 teeth were categorized into three groups at first, based on clinical outcomes (healthy, gingivitis, periodontitis). Subsequently, the periodontitis samples were further divided into three subgroups (early, moderate, and advanced periodontitis) according to the degree of periodontal attachment loss. Healthy and gingivitis were grouped as a reversible group, and the three subgroups were grouped as an irreversible group. To investigate trends of periodontopathic bacteria in the samples of dogs, a quantitative real-time polymerase chain reaction (PCR) was performed for quantification of 11 human periodontopathic bacteria as follows: Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Tannerella forsythia, Treponema denticola (Td), Fusobacterium nucleatum, Prevotella nigrescens, Prevotella intermedia, Parvimonas micra, Eubacterium nodatum, Campylobacter rectus, and Eikenella corrodens. The PCR results showed that Aa and Pg, the representative periodontopathic bacteria, were not significantly correlated or associated with the periodontitis cases in dogs. However, interestingly, Pi was strongly associated with the irreversible periodontal disease in dogs, in that it was the most prevalent bacterium detected from the dog samples. These findings indicate that the presence and numbers of Pi could be used as a prognostic biomarker in predicting the irreversible periodontal disease and the disease severity in dogs.

KEYWORDS: prognostic biomarker

DOI:
02.345/Sage.28.02.2025.01

1. INTRODUCTION

Periodontal disease is one of the most common disorders in the oral cavity of dogs and humans [1- 5]. Periodontal disease is a group of illness occurring in the supportive tissues of the teeth. There are two main types of periodontal disease, gingivitis and periodontitis, which are classified by disease severity and progression. Gingivitis, the initial stage of periodontal disease, is a reversible inflammatory condition that presents with swelling, redness, and bleeding from the gingiva. In contrast, periodontitis is a destructive and irreversible form of periodontal disease, resulting in tissue destruction in the periodontal ligament, cementum, alveolar bone, and gingiva [6]. Periodontitis caused by microbial biofilm often leads to host-mediated destruction of periodontal tissues, resulting in not only loss of teeth but also bloodstream bacterial infections [7]. It is therefore important to predict the prognosis of periodontal disease by identifying biomarkers that aid

in the development of appropriate therapeutic interventions and subsequently evaluating the periodontal tissues after the treatment.

Although the etiology of human periodontitis has been widely studied for several decades, the etiology of periodontal disease in dogs has not been completely explained yet. However, [6] evaluated putative pathogens related to periodontal disease in dogs The subgingival microbiota implicated in periodontal disease in dogs is predominantly constituted by gram-negative anaerobes, and it shows significant similarities to those of humans [6], [8], [9]. Another study has indicated that periodontal diseases in dogs are more closely related to those in humans than to other animals [10].

Although recent studies have found that the oral microbiome in dogs is significantly different from the human oral microbiome [11], [12], many previous studies have suspected that human periodontitis-related bacteria such as Porphyromonas gingivalis (Pg), Porphyromonas gulae, Prevotella intermedia (Pi), Fusobacterium nucleatum (Fn), Dialister pneumosintes, Actinobacillus actinomycetemcomitans, Campylobacter rectus (Cr), Eikenella corrodens (Ec), Tannerella forsythia (Tf), and Prevotella intermedia (Pi) are putative periodontitis-related pathogens in dogs [13], [14]. Most of these previous studies evaluated only the appearance of putative bacteria rather than trends in bacterial numbers and correlations of periodontitis stages in the dog. Furthermore, their limitations imposed by small cohort sizes have not been resolved [15], [16]. It is necessary to analyze trends in bacterial numbers and associations between bacterial species and various periodontal conditions in dogs to overcome the limitations of previous studies.

Throughout the present study, we simply put all the clinical samples into two classification groups largely. Those were the reversible group (collectively, the healthy and gingivitis sample group) and the irreversible periodontitis group. These two (reversible and irreversible) groups were basically used for the comparison with each other to assess the tested 11 bacteria that were thought to be associated with periodontal disease. A quantitative real-time polymerase chain reaction (PCR) was performed for the quantification of these 11 periodontopathic bacterial species as follows: Aggregatibacter actinomycetemcomitans (Aa), Pg, Tf, Td, Fn, Prevotella nigrescens (Pn), Pi, Parvimonas micra (Pm), Eubacterium nodatum (En), Cr, and Ec. Based on results of PCR analysis, the correlation and association between the number of bacterial species and various periodontal conditions were analyzed. For the first time in veterinary field, the current study also investigated the association between bacterial combination to understand the interrelationships of reversible and irreversible periodontal conditions with different severities.

2. Materials and methods

Sample collection sites were divided into rostral and caudal teeth of the oral cavity. Target teeth were mandibular and maxillary canine teeth known to be functionally important rostral teeth. The major masticatory teeth, maxillary fourth premolars teeth and mandibular first molars teeth, were also targeted as caudal teeth. Two samples were collected from each dog (one from the rostral target teeth and one from the caudal target teeth). One sample was collected if target teeth were missing in the oral cavity for some reasons.

All samples were obtained using a sterilized paper point International Organization for Standardization #30, which was gently inserted into the subgingival pocket at 6 points around each tooth for 30 seconds and transferred immediately into a sterilized transport tube. Six paper points obtained subgingival plaque from six points around a tooth, and all six paper points were placed in a sterilized sample container, then a unique barcode number was assigned to the sample container and analyzed as one sample. Teeth were examined and evaluated by intraoral dental radiography and periodontal probing.

ISSN: 18158129 E-ISSN: 18151027
Volume 21, Issue 02, February, 2025

Intraoral dental radiographs were obtained under general anesthesia and evaluated by the same veterinarian (DH-K) with a standard approach under the same conditions. According to the classification criteria of the American Veterinary Dental College [17], samples were divided into five PD groups based on clinical conditions, such as healthy (PD0), gingivitis (PD1), early periodontitis (PD2), moderate periodontitis (PD3), and advanced periodontitis (PD4). These five-stage PD grouping was based on the degree of attachment loss and gum condition. PD0 and PD1 included subjects without attachment loss. PD2, PD3, and PD4 included subjects with < 25%, 25%~50%, and > 50% attachment loss, respectively. A reversible group comprised PD0 and PD1, and an irreversible was group comprised PD2, PD3, and PD4.

DNA extraction

DNA extraction was performed using an ExgeneTM Cell SV kit (GeneAll Biotechnologies, Seoul, South Korea) according to the manufacturer's instructions. The paper point was treated with 180 µL lysozyme at 30 mg/mL and incubated at 37°C for 30 minutes. Proteinase K solution (20 µL of 20 mg/mL) and 200 µL of buffer BL were added to each sample followed by incubation at 56°C for 30 minutes and 95°C for 15 minutes. Subsequently, 200 µL of absolute ethanol was added, and the mixture was transferred into a column and centrifuged at 14,000 rpm for 1 minute. After washing the column with 600 µL of buffer BW, 700 µL of buffer TW was added. Next, 100 µL of buffer AE was used to elute DNA. DNA was quantified with a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). DNA samples were stored at –20°C before use.

Quantitative real-time polymerase chain reaction assay

Targeted oral bacteria and primer/probe set sequence used for quantitative real-time PCR in this study are listed in Table 1. PCR amplification was performed in a reaction volume of 20 µL (Bioneer, Inc., Daejeon, South Korea). PCR cycling was performed using a CFX96™ Real-Time System (Bio-Rad Laboratories Inc., Hercules, CA, USA). Cycling conditions consisted of an initial denaturation step at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, primer annealing at 60°C for 40 seconds, and primer extension at 72°C for 30 seconds. After completion of the cycling steps, a final extension step at 72°C for 5 minutes was performed. The normalized value of expression for each species was calculated as the ratio of the relative copy number of the reference species.

The and the data for assessment of specificity and sensitivity of the primer-probe sets detecting 11 bacteria from the subgingival plaques of dogs can be found in Supporting Information section.

Statistical methods

All statistical analyses were performed using the MedCalc Statistical Software version 18.5 (MedCalc Software, Ostend, Belgium). The independence of characteristics of samples was analyzed by independent t-tests. The correlation between the number of bacteria and the severity of the periodontal condition was evaluated by Pearson's correlation coefficient. Associations of each bacterium or combination of bacteria between reversible and irreversible groups were analyzed using logistic regression. The significance level was set at $p < 0.05$.

3. Results

Characteristic profiles of dogs that donated the subgingival plaque samples

Characteristic profiles of dogs that donated the subgingival plaque samples are summarized in Table 2. The mean ages were 9.36 and 9.69 years for the reversible and irreversible groups, respectively. Males accounted for 54.7% and 58.3% in the reversible and irreversible groups, respectively. The mean body weights were

6.44 kg and 5.79 kg for the reversible and irreversible groups, respectively. An independent t-test was performed to show a statistical independence between these characteristics of the dogs and the reversible and irreversible groupings based on the dog's periodontal disease conditions. The statistical results showed that these dog's characteristics were not significantly associated with their periodontal disease conditions. Of the total 336 samples, 190 samples (PD0, 70; PD1 120 samples) were classified into the reversible group, and 146 samples (PD2, 77; PD3, 34; PD4, 35 samples) were classified as the irreversible group.

The overall prevalence of the tested 11 bacteria detected in the reversible and irreversible groups is shown in Fig 1. Fn, Pm, and Ec showed a high prevalence over 50%, whereas Aa, Pg, and Pn showed a low prevalence less than 10%.

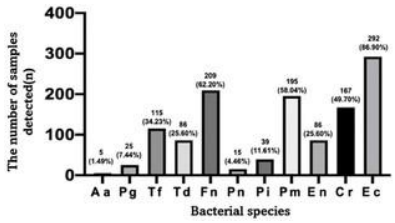

Fig 1. Overall prevalence of bacteria in the reversible and irreversible groups.

Bars represent the number of samples that each bacterial species was detected and the percentage of the number of samples detected to the number of total samples (%). Fn, Pm, and Ec showed high prevalence at over 50%, whereas Aa, Pg, and Pn showed low prevalence at less than 10%.

The prevalence of each bacterium was compared between the reversible and irreversible groups (Fig 2A). The prevalence of all bacterial species in the irreversible group was higher than that in the reversible group. Among the tested 11 bacterial species, Aa, Tf, Td, Pn, Pi, Pm, En, and Cr were detected two times more in the irreversible group compared with the reversible group.
Prevotella intermedia showed the biggest difference in prevalence among the reversible and irreversible groups at 5.95 times.
Treponema denticola showed the second biggest difference at 5.69 times.

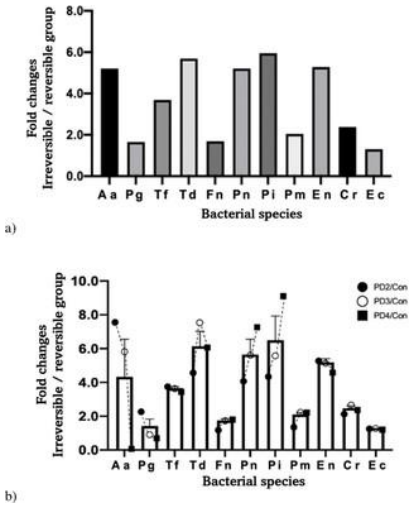

Fig 2. Prevalence of bacteria in comparison between groups.
(A) Comparison of fold changes between the reversible and irreversible groups. (B) Comparison of fold changes between the reversible group and each periodontal disease (PD) group of the irreversible group. PD2:

ISSN: 18158129 E-ISSN: 18151027
Volume 21, Issue 02, February, 2025

PD2 group, PD3: PD3 group, PD4: PD4 group, con: Reversible group.

This study also compared the prevalence of each bacterium between the reversible group and each PD group (Fig 2B). Although the severity of periodontal disease was getting worse from PD2 to PD4, the prevalence of Aa and Pg showed a gradually decreasing trend. In contrast, the prevalence of Fn, Pn, Pi, and Pm continually increased along the increase of PD severity.
Bacteria that showed increases over two times in their prevalence for all PD groups were Tf, Td, Pn, Pi, En, and Cr. were Tf, Td,
Pn, Pi, En, and Cr showed more than double the difference in the prevalence of all PD groups. The fold change of T. denticola, the biggest change, was 7.68 times between the reversible and PD3 groups. P. intermedia showed the biggest difference between the reversible and PD4 groups at 9.31 times. The bacterium that showed the biggest increase in its prevalence between the reversible group and PD3 group (moderate periodontitis) was T. denticola. P. intermedia showed the biggest difference between the reversible group and PD4 group (advanced periodontitis).

Mean number of bacteria

This study analyzed the mean number of all bacterial species in all groups (Fig 3). The mean number of F. nucleatum was 2.76E+07, which was the largest, whereas the mean number of A. actinomycetemcomitans was 6.21E+02, which was the smallest.

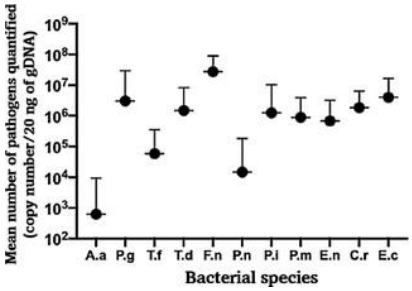

Fig 3. Overall mean numbers of bacteria in all groups. Bars show standard deviation. log = logarithm.

Mean numbers of bacteria in the reversible and irreversible groups.

Mean numbers of bacteria in the reversible and irreversible groups were compared. Results are shown in Fig 5A. Mean numbers of all bacterial species were increased in the irreversible group. Numbers of Aa, Pg, Td, Pm, En, and Cr in the irreversible group were higher over five times compared with those in the reversible group. However, Aa might not be associated with irreversible periodontal condition in dogs because of its extremely low overall bacterial number. The bacterium that showed the biggest difference in its number between the two groups was T. denticola (25.14 times). This result indicated a significant correlation between the number of bacteria and periodontitis, an irreversible condition.

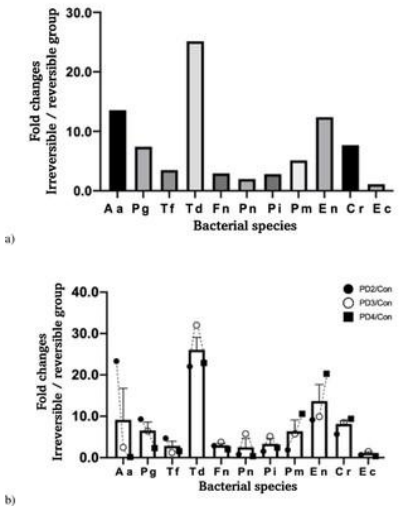

Fig 4. Mean numbers of bacteria in comparison between groups.
(A) Comparison of fold changes between the reversible and irreversible groups. PD2: PD2 group, PD3: PD3 group, PD4: PD4 group, con: Reversible group.

Mean numbers of bacteria in the reversible and PD groups.

Mean numbers of bacteria in the reversible group and each PD group were compared. Results are shown in Fig 4B. In the comparison between the reversible and PD2 groups, Aa and Td showed significant increases (24.31 times and 22.95 times, respectively). The difference of Td, 32.06 times, was unrivaled and overwhelming in comparison with the PD3 group. In the comparison of the reversible group and the PD4 group, Td and En showed the most overwhelming differences at 23.23 times and 21.62 times, respectively. Aa, Pg, and Tf showed significant differences in their numbers between the reversible and PD2 groups. However, the present study found that the number of bacteria continuously decreased as the severity progressed from PD3 to PD4. Specifically, Aa was not detected in the PD4 group. Conversely, Pm, En, and Cr increased continuously as severity progressed. Compared with the reversible group, the significant difference of Td in its number was observed in all PD groups. These results suggest that the number of bacteria except for a few could be correlated with the severity of periodontal condition, especially in the case of Prevotella i.

Correlation between the severity in the irreversible group and the number of bacterial species
Correlation analysis between the severity in the irreversible group and bacterial species was performed. All species, except Aa and Pg, showed reliable positive correlations with the irreversible group. In particular, Td, Pm, and Cr showed moderate correlations with Pearson's r > 0.4 (Table 1).

Species	Pearson's r	p-value
Aa	0.029	0.592
Pg	0.017	0.751
Tf	0.329	<0.001
Td	0.430	<0.001
Fn	0.357	<0.001
Pn	0.144	0.008
Pi	0.310	0.010
Pm	0.428	<0.001
En	0.354	<0.001
Cr	0.413	<0.001
Ec	0.260	0.006

Pearson's r: Pearson correlation coefficient.
Aa: Aggregatibacter actinomycetemcomitans, Pg: Porphyromonas gingivalis, Tf: Tannerella forsythia, Td: Treponema denticola, Fn: Fusobacterium nucleatum, Pn: Prevotella nigrescens, Pi: Prevotella intermedia, Pm: Parvimonas micra, En: Eubacterium nodatum, Cr: Campylobacter rectus, Ec: Eikenella corrodens.

https://doi.org/10.1371/journal.pone.0262859.t003

Table 1. Correlation between the severity of periodontal condition and the number of each bacterial species.

Association between the number of each bacterial species and periodontal condition

ISSN: 18158129 E-ISSN: 18151027
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Statistical methods were used to analyze associations between periodontal conditions and the number of bacteria using various comparisons (Table 2). Table 2 represents data that were $p > 0.05$. As shown in Table 2, the number of individual bacterial species was relatively increased, except for Aa and Pg, in the comparison between the reversible and irreversible groups. Similarly, Aa and Pg showed no significant reliability in associations between the reversible and each PD group. These facts indicated that the number of Aa and Pg had no association with periodontal conditions. Based on this, A. actinomycetemcomitans and P. gingivalis were excluded from further analysis.

Table 2. Relatively very low reliability of Aa and Pg in the Associations between the number of each bacterial species and the reversible and irreversible group and each PD group.

Association between the combinatory coexistence and numbers of bacterial species with the PD groups

With exception of Aa and Pg, as shown in Table 3, the combinatory coexistence and numbers of bacterial species among the remaining nine putative periodontopathic bacteria in the five-stage PD groups showed significant associations with the irreversible group and with the respective PD groups. For the comparison between the reversible and irreversible groups, bacterial numbers of Tf, Td, Pi, En, and Ec showed significant associations ($p = 0.001$, $p < 0.001$, $p = 0.006$, $p = 0.009$, and $p = 0.004$, respectively). In the case of comparison between the reversible group and the PD2 group, numbers of Tf, Td, En, and Ec ($p < 0.001$, $p = 0.001$, $p = 0.002$, and $p = 0.002$, respectively) showed significant associations with the PD2 group. For the comparison between the reversible and PD3 groups, numbers of Td, Fn, Pi, Pm, and Ec also showed significant associations with the PD3 group ($p < 0.001$, $p = 0.019$, $p = 0.001$, $p = 0.020$, and $p = 0.043$, respectively). Numbers of Td, Pi, and Pm also showed significant associations with the PD4 group in the comparison between the reversible and PD4 groups ($p = 0.005$, $p < 0.001$, and $p = 0.014$, respectively). Numbers of Td, Fn, Pi, and Pm showed significant associations with PD3 + PD4 group in the comparison between the reversible and PD3 + PD4 groups ($p < 0.001$, $p = 0.005$, $p < 0.001$, and $p = 0.004$, respectively).

	Species	Odd ratio (95% CI)	*p* value
Reversible group vs. irreversible group	*Tf*	1.109 (1.042–1.182)	0.001
	Td	1.159 (1.086–1.237)	<0.001
	Pi	1.105 (1.029–1.187)	0.006
	En	1.081 (1.019–1.146)	0.009
	Ec	1.161 (1.048–1.285)	0.004
Reversible group vs. PD2 group	*Tf*	1.138 (1.060–1.221)	<0.001
	Td	1.134 (1.050–1.224)	0.001
	En	1.114 (1.042–1.190)	0.002
	Ec	1.222 (1.078–1.386)	0.002
reversible group vs PD 3 group	*Td*	1.288 (1.156–1.435)	<0.001
	Fn	1.113 (1.018–1.352)	0.019
	Pi	1.207 (1.077–1.352)	0.001
	Pm	1.141 (1.021–1.276)	0.020
	Ec	1.283 (1.008–1.633)	0.043
Reversible group vs. PD4 group	*Td*	1.152 (1.043–1.273)	0.005
	Pi	1.195 (1.082–1.319)	<0.001
	Pm	1.145 (1.028–1.276)	0.014
Reversible group vs. PD3+4 group	*Td*	1.212 (1.117–1.315)	<0.001
	Fn	1.099 (1.029–1.175)	0.005
	Pi	1.193 (1.090–1.307)	<0.001
	Pm	1.127 (1.039–1.223)	0.004
PD2 group vs. PD3 group	*Td*	1.128 (1.039–1.226)	0.004
PD3 group vs. PD4 group			
PD2 group vs. PD4 group	*Pi*	1.097 (1.010–1.192)	0.028

Logistic regression.

https://doi.org/10.1371/journal.pone.0262859.t005

Table 3. Association between the combinatory coexistence and numbers of bacterial species with the periodontal disease groups.

P. intermedia showed a high significance in the comparison between the reversible and PD3 groups, between the reversible and PD4 groups, and between the reversible and PD3 + PD4 groups. However, T. forsythia showed a significance in the comparison between the reversible and PD2 groups only. Based on the above results, T. denticola seemed to be a superior species to others in all different comparisons analyzed statistically

in this study.

This study also statistically analyzed the association between each PD group. Only T. denticola was significantly associated with the PD3 group in the comparison between the PD2 and PD3 groups. P. intermedia was only significantly associated with the PD4 group in the comparison between the PD2 and PD4 groups. However, no bacteria showed significance in the comparison between the PD3 and PD4 groups.

4. References

[1] Albuquerque C, Morinha F, Requicha J, Martins T, Dias I, Guedes-Pinto H, et al. Canine periodontitis: the dog as an important model for periodontal studies. Vet J. 2012;191:299–305. pmid:21940182

[2] Kyllar M, Witter K. Prevalence of dental disorders in pet dogs. Vet Med (Praha). 2005;50:496–505.

[3] Harvey CE. Periodontal disease in dogs: etiopathogenesis, prevalence and significance. Vet Clin North Am Small Anim Pract. 1998;28:1111–1128. pmid:9779543

[4] Harvey CE. Small animal dentistry. Mosby-Year Books; 1993.

[5] Larsen J. Oral products and dental disease. Compend Contin Educ Vet. 2010;32:E4. pmid:20960410

[6] Yamasaki Y, Nomura R, Nakano K, Naka S, Matsumoto-Nakano M, Asai F, et al. Distribution of periodontopathic bacterial species in dogs and their owners. Arch Oral Biol. 2012;57:1183–1188. pmid:22417880

[7] Hirai N, Shirai M, Kato Y, Murakami M, Nomura R, Yamasaki Y, et al. Correlation of age with distribution of periodontitis-related bacteria in Japanese dogs. J Vet Med Sci. 2013;75:999–1001. pmid:23485527

[8] Holmstrom SE. Veterinary dentistry in senior canines and felines. Vet Clin North Am Small Anim Pract. 2012;42:793–808, viii. pmid:22720814

[9] Rawlinson JE, Goldstein RE, Reiter AM, Attwater DZ, Harvey CE. Association of periodontal disease with systemic health indices in dogs and the systemic response to treatment of periodontal disease. J Am Vet Med Assoc. 2011;238:601–609. pmid:21355802

[10] Senhorinho GN, Nakano V, Liu C, Song Y, Finegold SM, Avila-Campos MJ. Occurrence and antimicrobial susceptibility of Porphyromonas spp. and Fusobacterium spp. in dogs with and without periodontitis. Anaerobe. 2012;18:381–385. pmid:22609780

[11] Davis IJ, Wallis C, Deusch O, Colyer A, Milella L, Loman N, et al. A cross-sectional survey of bacterial species in plaque from client owned dogs with healthy gingiva, gingivitis or mild periodontitis PLoS One. 2013;8:e83158. pmid:24349448

[12] Wallis C, Marshall M, Colyer A, O'Flynn C, Deusch O, Harris S. A longitudinal assessment of changes in bacterial community composition associated with the development of periodontal disease in dogs. Vet Microbiol. 2015;181:271–282. pmid:26507828

[13] Kato Y, Shirai M, Murakami M, Mizusawa T, Hagimoto A, Wada K, et al. Molecular detection of human periodontal pathogens in oral swab specimens from dogs in Japan. J Vet Dent 2011;28:84–89. pmid:21916371

[14] Nishiyama SAB. Detection of putative periodontal pathogens in subgingival specimens of dogs. Brazilian J Microbiol. 2007;38:23–28.

[15] Dahlen G, Charalampakis G, Abrahamsson I, Bengtsson L, Falsen E. Predominant bacterial species in subgingival plaque in dogs. J Periodontal Res. 2012;47:354–364. pmid:22181039

[16] Riggio MP, Lennon A, Taylor DJ, Bennett D. Molecular identification of bacteria associated with canine periodontal disease. Vet Microbiol. 2011;150:394–
400. pmid:21489726

[17] AVDC: Nomenclature stage of periodontal disease. 2017. Available from: https://www.avdc.org/avdc-nomenclature/).

[18] Nonnenmacher C, Dalpke A. Mutters R, Heeg K. Quantitative detection of periodontopathogens by real-time PCR. J Microbiol Methods. 2004;59:117–125. pmid:15325758

[19] Price RR, Viscount HB, Stanley MC, Leung KP. Targeted profiling of oral bacteria in human salica and in vitro biofilms with quantitative real-time PCR. Biofouling. 2007;23:203–213. pmid:17653931

[20] Dewhirst FE, Klein EA, Thompson EC, Blanton JM, Chen T, Milella L, et al. The canine oral microbiome. PLoS One. 2012;7:e36067. pmid:22558330

he development of periodontal disease in dogs. Vet Microbiol. 2015;181:271–282. pmid:26507828 View Article PubMed/NCBI Google Scholar

[22] Kumar PS, Griffen AL, Moeschberger ML, Leys EJ. Identification of candidate periodontal pathogens and beneficial species by quantitative 16S clonal analysis. J Clin Microbiol. 2005;43:3944–3955. pmid:16081935

[23] Griffen AL, Beall CJ, Campbell JH, Firestone ND, Kumar PS, Yang ZK, et al. Distinct and complex bacterial profiles in human periodontitis and health revealed by 16S pyrosequencing. ISME J. 2012;6:1176–1185. pmid:22170420

[24] Di Bello A, Buonavoglia A, Franchini D, Valastro C, Ventrella G, Greco MF,. Periodontal disease associated with red complex bacteria in dogs. J Small Anim Pract. 2014;55:160–163. pmid:24450418

[25] Özavci V, Erbas G, Parin U, Yüksel HT, Kirkan Ş. Molecular detection of feline and canine periodontal pathogens. Vet Anim Sci. 2019;8:100069. pmid:32734086

[26] Polkowska I, Sobczyńska-Rak A, Gołyńska M. Analysis of gingival pocket microflora and biochemical blood parameters in dogs suffering from periodontal disease. In Vivo. 2014;28:1085–1090. pmid:25398803

[27] Watanabe T, Hara Y, Yoshimi Y, Fujita Y, Yokoe M, Noguchi Y. Clinical characteristics of bloodstream infection by Parvimonas micra: retrospective case series and literature review. BMC Infect Dis. 2020;20:578. pmid:32758181

[28] Socransky SS, Haffajee AD. Periodontal microbial ecology. Periodontol 2000. 2005;38:135–187. pmid:15853940 View Article PubMed/NCBI Google Scholar

[29] Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25:134–144. pmid:9495612

[30] Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen hypothesis. Nat Rev Microbiol. 2012;10:717–725. pmid:22941505 View Article PubMed/NCBI Google Scholar

[31] Hajishengallis G, Lamont RJ. Dancing with the stars: how choreographed bacterial interactions dictate nososymbiocity and give rise to keystone pathogens, accessory pathogens, and pathobionts. Trends Microbiol. 2016;24:477–489. pmid:26968354

[32] Fine DH, Patil AG, Velusamy SK. Aggregatibacter actinomycetemcomitans under the radar: myths and misunderstandings of Aa and its role in aggressive periodontitis. Front Immunol 2019;10:728. pmid:31040843

[33] Nises J, Rosander A, Pettersson A, Backhans A. The occurrence of Treponema spp. in gingival plaque from dogs with varying degree of periodontal disease. PLoS One. 2018;13:e0201888. pmid:30092089

[34] Senhorinho GN, Nakano V, Liu C, Song Y, Finegold SM, Avila-Campos MJ. Detection of Porphyromonas gulae from subgingival biofilms of dogs with and without periodontitis. Anaerobe. 2011;17:257–258. pmid:21723404

[35] Fournier D, Mouton C, Lapierre P, Kato T, Okuda K, Menard C. Porphyromonas gulae sp. nov., an anaerobic, gram-negative coccobacillus from the gingival sulcus of various animal hosts. Int J Syst Evol Microbiol. 2001;51:1179–1189. pmid:11411686

[36] Hamada N, Takahashi Y, Watanabe K, Kumada H, Oishi Y, Umemoto T. Molecular and antigenic similarities of the fimbrial major components between Porphyromonas gulae and P. gingivalis. Vet Microbiol. 2008;128:108–117. pmid:17977673