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Distribution of periodontopathic bacterial species in dogs and their owners

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ABSTRACT

Objective: Presently, a large number of individuals consider their companion animals as family members and have close contact with them in daily life. The purpose of the present study was to analyze the distribution of periodontopathic bacterial species in oral specimens taken from dogs and their owners.

Design: Dental plaque specimens were collected from 66 dogs and 81 members of 64 families who came to an animal clinic or dog training school in Okayama, Japan, in 2011. Bacterial DNA was extracted from each specimen and PCR analyses using primers specific for 11 periodontopathic species, *Porphyromonas gingivalis*, *Porphyromonas gulae*, *Treponema denticola*, *Tannerella forsythia*, *Capnocytophaga ochracea*, *Capnocytophaga sputigena*, *Prevotella intermedia*, *Prevotella nigrescens*, *Aggregatibacter actinomycetemcomitans*, *Campylobacter rectus*, and *Eikenella corrodens* were performed.

Results: *P. gulae* (71.2%), *T. forsythia* (77.3%), and *C. rectus* (66.7%) were frequently found in the dogs, whereas the detection rates of those species in humans were less frequent at 16.0%, 30.9%, and 21.0%, respectively. *P. gulae* was identified in 13 human subjects and each of their dogs was also positive for the species. Furthermore, *E. corrodens* and *T. denticola* in specimens obtained from dogs were correlated with their presence in specimens from owners who had close contact with them.

Conclusions: These results suggest that several periodontopathic species could be transmitted between humans and their companion dogs, though the distribution of periodontopathic species in both is generally different.

1. Introduction

Dental caries and periodontitis are two major diseases in humans, both of which are caused by specific oral pathogens.[1,2] Recently, greater numbers of individuals consider their companion animals as family members and have close contact with them in daily life. It is known that dental caries is quite uncommon in dogs, with a prevalence of only 5%, which is possibly derived from the relatively alkaline pH canine oral environment.[3] On the other hand, the prevalence rates of gingivitis and periodontitis in dogs were reported to range from 95–100% and 50–70%, respectively.[4] However, there is limited information regarding the oral microbiota in dogs.[5] We recently

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analyzed the distribution of 10 human periodontitis-related species in client-owned dogs, and found that *Tannerella forsythia* and *Campylobacter rectus* were detected with extremely high frequency.[6] In addition, *Porphyromonas gulae*, uncommon in the human oral cavity was found to be one of the major species detected in dogs.[6]

It is widely accepted that transmission of oral bacteria including pathogens related to dental caries and periodontitis occurs between mothers and their children by close contact in daily life.[7,8] Thus, it is possible that transmission of oral bacteria between humans and their companion animals could also occur when they have routine close contact. To our knowledge, few studies have analyzed the distribution of oral bacteria in humans and their companion animals, though a recent comparison of periodontopathic species in cats and their owners revealed that *T. forsythia* may be one of the species transmitted between them.[9] In the present study, we investigated the prevalence of periodontopathic species in dogs and their owners using oral specimens subjected to a molecular biological method to investigate the possibility of owner–pet transmission of periodontopathic bacteria.

2. Methods

2.1. Subjects

We studied 81 dog owners (19 males, 62 females) from 64 families and their 66 dogs (27 males, 39 females). All study protocols were approved by the Animal Research Committee of Azabu University, and the Ethics Committees of Osaka University Graduate School of Dentistry and Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences. All specimens were collected at a single animal clinic and a single dog training school in Okayama, Japan, from October 2011 to December 2011. Prior to collection, the owners were informed of the purpose of the present study and gave approval for their participation. None of the dogs or their owners received antibiotics treatment within the previous 3 months prior to sample collection. The dogs ranged in age from 1 to 13 years of old (median, 6 years) (Fig. 1) and the different breeds are summarized in Table 1.

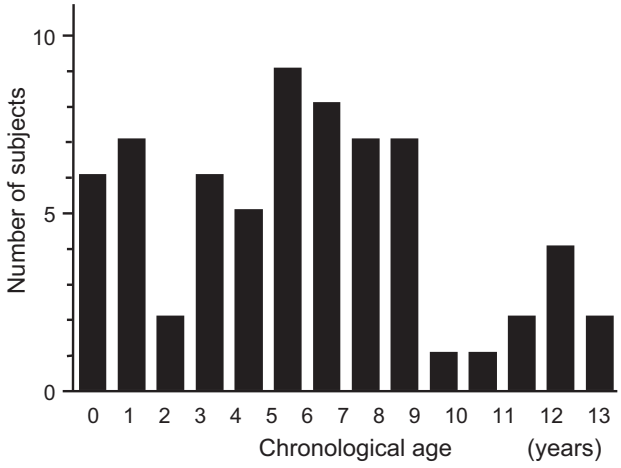

Fig. 1 – Age distribution of dogs in the present study.

Table 1 – Dog breeds in the present study.

Breed	Numbers (male:female)	Age (median)
Miniature Dachshund	12 (5:7)	1–11 (5.5)
Toy poodle	9 (4:5)	0–8 (1)
Mix	7 (2:5)	4–13 (5)
Chihuahua	6 (2:4)	0–7 (5.5)
Collie	4 (0:4)	5–6 (5)
Corgi	3 (3:0)	0–3 (0)
Miniature Schnauzer	3 (3:0)	1–6 (6)
Pomeranian	3 (1:2)	4–12 (8)
Yorkshire Terrier	3 (1:2)	7–10 (8)
Others[a]	16 (7:9)	1–12 (6)
Total	66 (28:38)	0–13 (5)

[a] Others include Shiba, Labrador Retriever, Bull Terrier, Norfolk Terrier, Cavalier King Charies Spniel, Saint-Bernard, Maltese, Jack Russell, Shih Tzu and Border Collie.

Contact between the dogs and their owners were defined as follows: 0, no contact (living in places different from each other) ($n = 1$ owner), 1A, nearly no contact with dog kept outdoors ($n = 4$), 1B: nearly no contact with dog kept indoors ($n = 7$), 2A: frequent contact with dog kept outdoors ($n = 6$), and 2B, frequent contact with dog kept indoors ($n = 63$).

2.2. Collection of oral specimens and extraction of bacterial DNA

Dental plaque specimens from the dogs and their owners were collected from the gingival margin of the buccal side of the maxillary left molar region using sterile toothpicks, which were then dispersed in sterile distilled water in sterile plastic tubes. Next, the specimens were centrifuged and the sediment was suspended in sterile saline. Bacterial DNA of each specimen was extracted using a Puregene Yeast/Bact. Kit B (QIAGEN Inc., Valencia, CA, USA), according to the method described by the manufacturer.

2.3. Molecular detection for periodontitis-related species

PCR was performed to identify bacterial DNA for 11 periodontopathic species using bacterial DNA extracted from the specimens with species-specific sets of primers (Table 2). A universal primer set was used to confirm that bacterial DNA was appropriately extracted.[10] We targeted the following 10 species reported to be associated with periodontitis in humans after analysis of previous reports: *Porphyromonas gingivalis*, *Treponema denticola*, *T. forsythia*, *Capnocytophaga ochracea*, *Capnocytophaga sputigena*, *Prevotella intermedia*, *Prevotella nigrescens*, *Aggregatibacter actinomycetemcomitans*, *C. rectus*, and *Eikenella corrodens*.[11–14] Specificity with this method was previously shown in those studies, with the minimum detection level for the species reported to be 10–100 cells. In addition to those 10 species, we tested for *P. gulae*, which is known to be isolated from the gingival sulcus of various animal hosts and is distinct from related strains of *P. gingivalis* of human origin.[6,15]

2.4. Statistical analyses

Statistical analyses were performed using the computational software packages StatView 5.0 (SAS Institute Inc., Cary, NC,

Table 2 – PCR primer sets used for detection of 11 periodontopathic bacterial species.

Target species	Sequence (5′ to 3′)	Size (bp)	References
Universal primer (positive control)	AGA GTT TGA TCM TGG CTC AG CTG CTG CSY CCC GTA G	315	[10]
Porphyromonas gingivalis	CCG CAT ACA CTT GTA TTA TTG CAT GAT ATT AAG AAG TTT ACA ATC CTT AGG ACT GTC T	267	[6]
Treponema denticola	AAG GCG GTA GAG CCG CTC A AGC CGC TGT CGA AAA GCC CA	311	[11]
Tannerella forsythia	GCG TAT GTA ACC TGC CCG CA TGC TTC AGT GTC AGT TAT ACC T	641	[12]
Capnocytophaga ochracea	AGA GTT TGA TCC TGG CTC AG GAT GCC GTC CCT ATA TAC TAT GGG G	185	[13]
Capnocytophaga sputigena	AGA GTT TGA TCC TGG CTC AG GAT GCC GCT CCT ATA TAC CAT TAG G	185	[13]
Prevotella intermedia	TTT GTT GGG GAG TAA AGC GGG TCA ACA TCT CTG TAT CCT GCG T	575	[12]
Prevotella nigrescens	ATG AAA CAA AGG TTT TCC GGT AAG CCC ACG TCT CTG TGG GCT GCG A	804	[12]
Campylobacter rectus	TTT CGG AGC GTA AAC TCC TTT TC TTT CTG CAA GCA GAC ACT CTT	598	[12]
Aggregatibacter actinomycetemcomitans	CTA GGT ATT GCG AAA CAA TTT G CCT GAA ATT AAG CTG GTA ATC	262	[14]
Eikenella corrodens	CTA ATA CCG CAT ACG TCC TAA G CTA CTA AGC AAT CAA GTT GCC C	688	[12]
Porphyromonas gulae	TTG CTT GGT TGC ATG ATC GG GCT TAT TCT TAC GGT ACA TTC ACA	314	[6]

USA) and Prism 4 (GraphPad Software Inc., San Diego, CA, USA). Regression analysis was performed to compare the total numbers of bacterial species and chronological ages of the dogs for each specimen. An unpaired student's *t*-test was used for comparisons of total numbers of bacterial species in the *P. gulae*-positive and -negative groups. Fisher's protected least-significant difference test was utilized to compare the detection frequencies of *T. forsythia* and *C. rectus*. Odds ratio (OR) and 95% confidence interval (CI_{95}) values were calculated to determine any significant association regarding the presence of *P. gulae*, *T. forsythia*, and *C. rectus*. Fisher's protected least-significant difference test was also used to analyze the relationship of bacterial species between dogs and their owners, while OR and CI_{95} values were calculated to determine any significant association between the presence of each species in dogs and owners. The results were considered significantly different with *P* values of less than 0.05.

3. Results

T. forsythia was the most frequently detected species in dogs with a prevalence of 77.3%, while it was detected in only 30.9% of the owners (Fig. 2). The detection rates of *C. rectus* and *P. gulae* in dogs were 66.7% and 71.2%, respectively, whereas those were detected in approximately 15–20% of the owners. *E. corrodens* was identified in both dogs and owners, with a prevalence of approximately 20–25%. *C. sputigena* was identified in 33.3% and *C. ochracea* in 9.9% of the owners, though both were rarely found in specimens obtained from dogs. The detection rate of *P. nigrescens* in owners was 18.5%, and that in dogs was 7.6%. *P. gingivalis*, *T. denticola*, *P. intermedia* and *A. actinomycetemcomitans* were rarely found in either dogs or their owners.

The total numbers of bacterial species in dogs showed a significantly positive correlation with their chronological age (Fig. 3; $P = 0.0041$). *P. gulae* was identified in approximately 70% of all dogs and the total numbers of bacterial species in the *P. gulae*-positive group was significantly greater as compared to the *P. gulae*-negative group (Fig. 4A; $P < 0.001$). In addition, the detection rate for both *T. forsythia* and *C. rectus* in the *P. gulae*-positive group approximately was 80%, which was significantly greater than that in the *P. gulae*-negative group by almost four-fold (Fig. 4B; $P < 0.001$). The OR values for identification of *T. forsythia* and *C. rectus* in *P. gulae*-positive specimens were 6.15 and 9.15, respectively, while that of *T. forsythia* in *C. rectus*-positive specimens was 30.33 (Fig. 4C; $P < 0.001$).

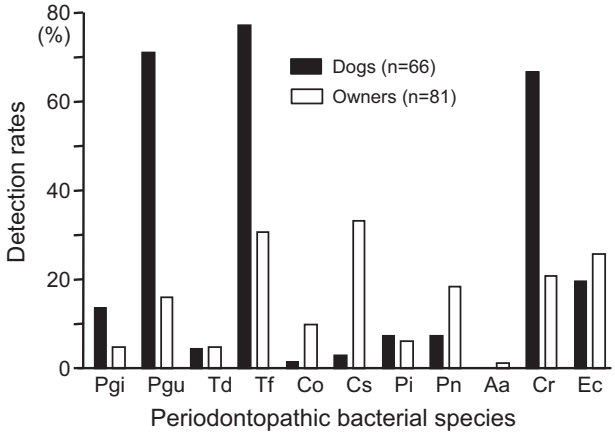

Fig. 2 – Distribution of periodontopathic bacteria in dogs and their owners. Pgi, *Porphyromonas gingivalis*; Pgu, *P. gulae*; Td, *Treponema denticola*; Tf, *Tannerella forsythia*; Co, *Capnocytophaga ochracea*; Cs, *Capnocytophaga sputigena*; Pi, *Prevotella intermedia*; Pn, *Prevotella nigrescens*; Aa, *Aggregatibacter actinomycetemcomitans*; Cr, *Campylobacter rectus*; Ec, *Eikenella corrodens*.

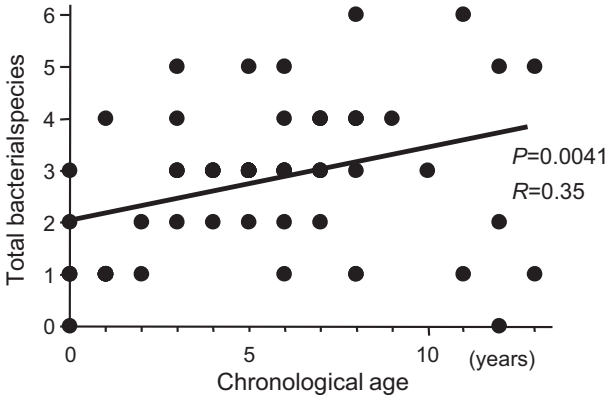

Fig. 3 – Correlations of age and total numbers of bacterial species in dogs. Results of regression analysis are shown. Each dot indicates an individual specimen.

P. gulae was detected in 13 owners (1 person with contact score 1A, 1 person with contact score 1B, 1 person with contact score 2A, 10 persons with contact score 2B) and all of their dogs showed a positive reaction to *P. gulae*. There were 2 families who kept two dogs in the same house, whose bacterial profiles are totally consistent each other (Fig. 5). The bacterial profiles of dogs and their owners were compared based on the classification of contact scores. *E. corrodens* was significantly correlated in the dogs and their owners with close contact with dogs (Table 3). In addition, the *P* values of *P. gulae* and *T. denticola* were close to 0.05 in the group with contact scores of 2. On the other hand, there were no species with correlation in dogs and their owners in groups with contact score of 1 or 0 (nearly no or no contact, respectively).

4. Discussion

To our knowledge, this is the first study to analyze the distribution of specific periodontitis-related pathogens in dental plaque specimens taken from both dogs and their owners using a molecular biological approach. In our previous study, we investigated the distribution of 10 periodontitis-related species using a method designed for the analyses of human specimens and found that specific species, such as *T. forsythia* and *C. rectus*, were frequently identified in dental plaque specimens collected from dogs.[6] In addition, *P. gulae* was reported to be frequently found in specimens from dogs, though it is regarded as quite uncommon in humans.[15] These findings led us to consider that there are several species predominantly harboured by dogs, and focused on the distribution of bacterial species in dogs and their owners in the present study.

Transmission of human oral bacterial species from mothers to their children has been widely investigated.[16] Our previous analysis using a molecular biological technique showed that 70% of *Streptococcus mutans* strains, a pathogen of dental caries, in children under the age of 10 were transmitted from their mothers, possibly because of close contact during daily life.[8] In addition, periodontitis-related species such as *T. denticola* were frequently detected in children whose parents possessed the same pathogen.[7] These findings led us to consider the possibility that some species could be transmitted between humans and dogs when they had close contact. In fact, the present study showed that the presence of *E. corrodens*, and possibly *P. gulae* and *T. denticola*, in dogs and their owners is correlated. In addition, it is interesting that the same bacterial profiles were identified in two dogs kept in the same house. Furthermore, the same bacterial profiles were also identified in two dogs living next door who played frequently with the pair (data not shown). This finding indicates that the transmission of peridontopathic bacteria could occur between dogs under the condition of close contact. Further studies should be focused on this possibility.

Transmission of bacterial strains between subjects should be directly investigated by more appropriate methods, such as genotyping of the isolated bacteria. However, isolation of periodontopathic species is quite difficult and time consuming, as most are classified as obligate anaerobic organisms. On

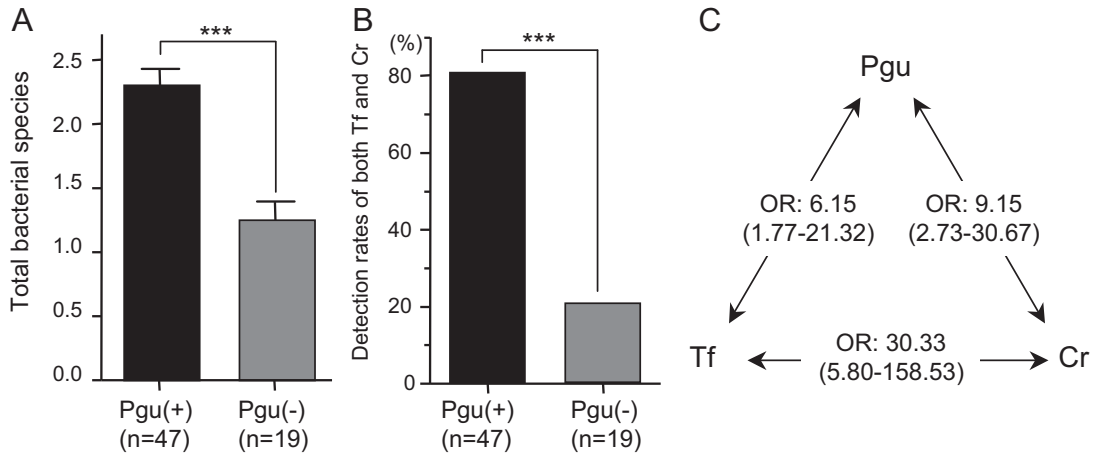

Fig. 4 – Comparisons of *P. gulae*-positive and -negative groups. (A) Total number of bacterial species except for *P. gulae*. (B) Detection rates of *T. forsythia* and *C. rectus*. Statistically significant differences were identified (*P < 0.001). (C) Bacterial species coexisting in saliva specimens of dogs. Pgu, *P. gulae*; Tf, *T. forsythia*; Cr, *C. rectus*; OR, odds ratio. Values in parentheses indicate the 95% confidence interval.**

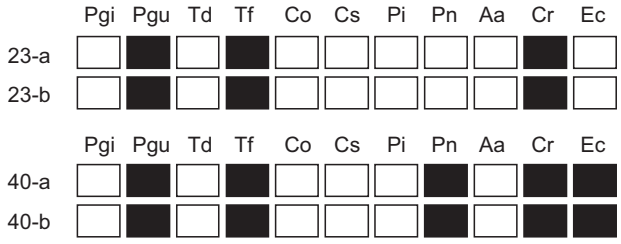

Fig. 5 – Bacterial profiles in 2 dogs kept in the same family. Closed and open rectangles indicate a positive and negative reaction, respectively, to corresponding species for 5-year-old (23-a) and 3-year-old (23-b) female dogs kept in the same house, 6-year-old (40-a) and 5-year-old (40-b) female dogs kept in the same house.

the other hand, the molecular biological technique used in the present study may be a better choice for large-scale studies. Therefore, we performed species-specific PCR analyses and determined the presence of various species in the obtained specimens in order to determine whether some may have been transmitted between dogs and their owners.

It is generally accepted that saliva and dental plaque specimens reflect the whole oral cavity and specific localized areas, respectively.[7] We consider that it would be better to collect saliva specimens from both humans and dogs in the present study. However, collection of saliva from dogs is quite difficult. If saliva specimens were obtained from dogs and analyzed, it is possible that more species would have been detected. Thus, large scale studies are needed to more clearly specify bacterial transmission between dogs and humans.

The subjects of the present studies were selected from owners who came to an animal clinic or dog training school. Thus, the results are limited by the absence of clinical data for periodontitis in the dogs and owners. In general, individuals and dogs with periodontitis harbour various types of periodontopathic species in the oral cavity.[1] It is possible to speculate that those who possess greater numbers of species have worse periodontal conditions. The present study indicated that the total number of species detected in the dogs was positively correlated with their chronological age. It has been reported that susceptibility to periodontitis increases with age in both dogs and humans.[1,4] Thus, analysis of older dogs and humans is needed to more precisely clarify the transmission of bacterial species between them.

The prevalence of *P. gulae* in dental plaque specimens obtained from dogs with and without periodontitis was reported to be 92% and 56%, respectively.[17] In our previous study, the frequency of *P. gulae* was more than 90% in oral specimens collected from dogs at an animal clinic in Tokyo.[6] In contrast, *P. gulae* was identified in 71.2% of all dogs in the present study, which could be regarded as low. The dog training school that participated in the present study focuses on oral health, which may have led to the better periodontal conditions seen in the dog subjects. Additional studies that evaluate changes in periodontopathic bacteria in dogs with interventions such as professional oral care and oral health instructions to the owners are also needed.

It is interesting that *P. gulae* was detected in 13 of the owner specimens (16.0%), though this species is regarded to be uncommon in the human oral cavity.[15] In addition, *P. gulae* was detected in all of the dogs kept by those 13 owners, indicating that the organisms can be transmitted from dogs to humans. It should be noted that the contact scores of two individuals were 1A and 1B each, indicating that this species could be transmitted even by with less frequent contact with dogs. Therefore, further studies that focus on this possibility are important. In the present study, the presence of *E. corrodens* and possibly *T. denticola* in humans was shown to be correlated with that in their dogs with whom they had close daily contact. Approximately 85% of the present human subjects had close contact with their dogs, which limited our analysis because of the relatively few owners and dog pairs without close contact.

In summary, we found that the distribution of periodontopathic bacterial species in dogs and their owners is diverse, though several species including *P. gulae* may be transmitted during close daily contact. Therefore, our findings could be significant in understanding the relationship between the oral health of humans and their companion animals.

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Table 3 – Correlation of presence of periodontopathic bacteria in dogs and their owners based on contact scores.

Species	Contact score 2 ($n = 69$)		Contact score 1 or 0 ($n = 12$)	
	P values	Odds ratio (CI95)	*P* values	Odds ratio (CI95)
E. corrodens	0.0107	5.03 (1.48–17.12)	0.3182	–
T. denticola	0.0556	10.00 (1.28–78.12)	1.0000	–
P. gulae	0.0549	–	1.0000	–
P. intermedia	0.3216	–	1.0000	–
C. rectus	0.5339	–	0.4545	–
P. gingivalis	0.5938		1.0000	–
T. forsythia	0.7203	–	0.5454	–
C. ochracea	1.0000	–	1.0000	–
C. sputigena	1.0000	–	1.0000	–
P. nigrescens	1.0000	–	1.0000	–
A. actinomycetemcomitans	1.0000	–	1.0000	–

Competing interests

None.

Ethical approval

All study protocols were approved by the Animal Research Committee of Azabu University, and the Ethics Committees of Osaka University Graduate School of Dentistry and Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences.

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