NOTE *Bacteriology*

Correlation of Age with Distribution of Periodontitis-Related Bacteria in Japanese Dogs

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ABSTRACT. We analyzed the distribution of 11 periodontitis-related bacterial species in dental plaque collected from 176 Japanese dogs divided into young (less than 2 years of age), middle-aged (2–7 years of age) and elderly (more than 8 years of age) groups using a polymerase chain reaction method. Clinical examination revealed that no dogs in the young group were affected by periodontitis, whereas the rates for gingivitis and periodontitis were high in the middle-aged and elderly groups. In addition, the total numbers of bacterial species in the middle-aged and elderly groups were significantly greater than in the young group. Our findings suggest that age is an important factor associated with the distribution of periodontitis-related bacteria and periodontal conditions in dogs.
KEY WORDS: age, canine, dental plaque, periodontitis, periodontitis-related bacteria.

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Periodontal diseases are quite commonly identified in dogs. The diseases are composed of gingivitis, a reversible condition that features limited inflammation of gingival tissue, and periodontitis, which indicates destruction of periodontal tissues, such as the cementum, periodontal ligaments and supportive bone [8]. It has been summarized that the distribution frequency of gingivitis in dogs aged from 0 to 14 years old (average; approximately 6 years old) ranges from 95–100%, while that of periodontitis is from 50–70% [3].

Our previous study analyzed the distribution of 10 human periodontitis-related species in dogs and found that specific species, such as *Tannerella forsythia* and *Campylobacter rectus*, were frequently detected [5]. In addition, *Porphyromonas gulae*, a major pathogen of periodontitis in dogs [2], was also detected at a high frequency [5]. On the other hand, the detection rates of other species, including *Porphyromonas gingivalis*, *Treponema denticola*, *Capnocytophaga ochracea*, *Capnocytophaga sputigena*, *Prevotella intermedia*, *Prevotella nigrescens*, *Aggregatibacter actinomycetemcomitans* and *Eikenella corrodens*, were quite low [5]. In addition, several periodontopathic species were shown to be possibly transmitted between dogs and their owners, although the distribution of periodontopathic species in both is generally different [12].

In the present study, to investigate the influence of the age of dogs on the periodontal conditions and the distribution of the periodontitis-related species, 176 Japanese dogs were analyzed. On the basis of the age classifications of young (less than 2 years of age), middle-aged (2–7 years of age) and elderly (more than 8 years of age) dogs [6], the 176 dogs were classified into the young (n=39), middle-aged (n=62) and elderly (n=75) groups. The protocols used in this study were approved by Azabu University, Osaka University Graduate School of Dentistry and Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences. The owners of the dogs also approved their participation in this study.

Periodontal condition was evaluated by measuring several parameters of a representative tooth (mandibular left canine), as previously described [13]. Briefly, periodontal pocket depth was measured to the nearest millimeter around the circumference of each tooth from the gingival margin to the deepest probing point, using a round-ended probe tip 0.4 mm in diameter, with the maximum value recorded. A periodontal pocket depth of 3 mm or less for large dogs, and 2 mm or less for medium and small dogs was regarded as healthy. In addition, no bleeding on probing, no pus discharge and no tooth mobility were regarded as healthy criteria. When deeper periodontal pockets and/or bleeding on probing were observed, the dogs were diagnosed with gingivitis or periodontitis. Periodontitis was diagnosed by pathological mobility due to obvious destruction of periodontal tissues, such as alveolar bone.

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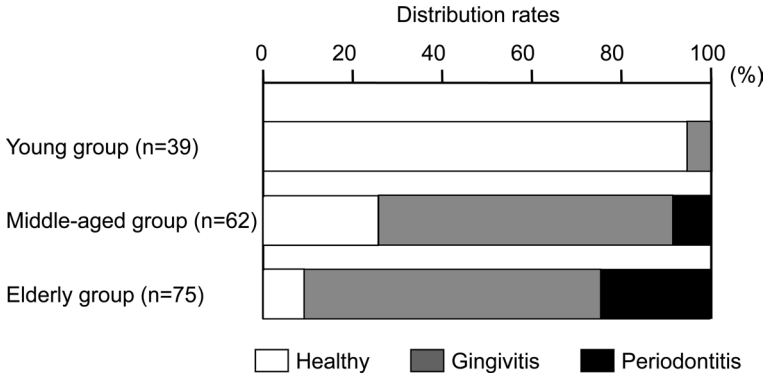

Fig. 1. Distribution rates of periodontal conditions in each age group.

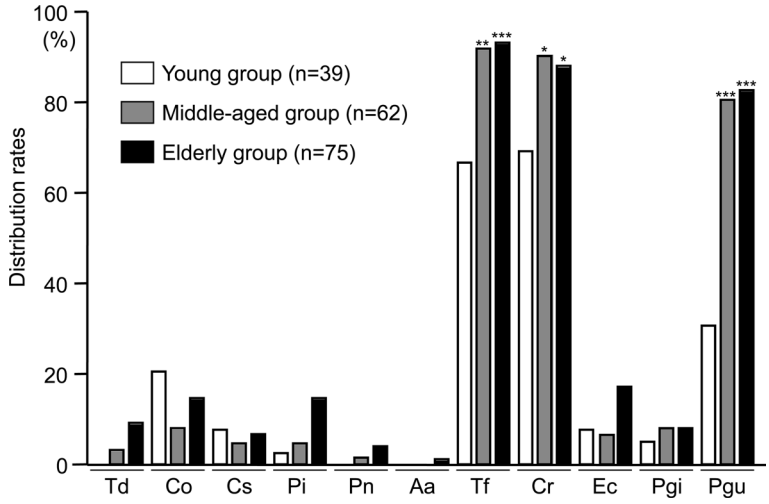

Fig. 2. Distribution rates of 11 periodontitis-related species in dental plaque specimens collected from each group. There were statistically significant differences in the group (**P*<0.05, ***P*<0.01, ****P*<0.001). Td; *T. denticola*, Co; *C. ochracea*, Cs; *C. sputigena*, Pi; *P. intermedia*, Pn; *P. nigrescens*, Aa; *A. actinomycetemcomitans*, Tf; *T. forsythia*, Cr; *C. rectus*, Ec; *E. corrodens*, Pgi; *P. gingivalis* and Pgu; *P. gulae.*

Figure 1 shows the distribution rates of dogs classified as healthy, with gingivitis and with periodontitis in each group. Fisher's protected least-significant difference test was utilized to compare the groups. None of the dogs in the young group were affected by periodontitis, whereas the distribution rate in the elderly group (24.0%) was significantly elevated compared to the young dogs ($P<0.001$). In addition, the distribution rate of gingivitis in the middle-aged and elderly groups (67.7% and 66.7%, respectively) was significantly greater than in the young group (5.1%) ($P<0.001$), while the distribution rate of dogs with a healthy periodontal condition was significantly higher in the young group (94.9%) than in the middle-aged and elderly groups (25.8% and 9.3%, respectively) ($P<0.001$). Harvey *et al*. [3] previously reported that the periodontal conditions in older dogs are worse than those in younger dogs in North America. Our present data suggested that age is also an important factor associated with the periodontal conditions in Japanese dogs.

Periodontitis-related species in dental plaque specimens were detected as previously described [5]. Briefly, oral specimens were collected from a specific location, the gingival margin of the mandibular left canine in the oral cavity, using swabs (Seed-Swab γ-1 or 2; Eiken Chemical Co., Ltd., Tokyo, Japan). Then, bacterial DNA was extracted using a Puregene Yeast/Bact. Kit B (QIAGEN Inc., Valencia, CA, U.S.A.). PCR was performed to identify bacterial DNA for the following 11 periodontopathic species, *T. denticola*, *C. ochracea*, *C. sputigena*, *P. intermedia*, *P. nigrescens*, *A. actinomycetemcomitans*, *T. forsythia*, *C. rectus*, *E. corrodens*, *P. gingivalis* and *P. gulae*, using bacterial DNA extracted from the specimens with species-specific sets of primers.

The distribution frequency of the 11 periodontitis-related species was statistically analyzed using Fisher's protected least-significant difference test to compare them among

the groups. The detection rates of *P. gulae*, *T. forsythia* and *C. rectus* were significantly higher in the middle-aged and elderly dogs than in the young dogs ($P<0.001$, $P<0.01$ and $P<0.05$, respectively) (Fig. 2). On the other hand, the distribution rates of other species were not significantly different among the groups. *P. gulae* is closely related to *P. gingivalis*, one of the major periodontitis pathogens in humans [1]. Furthermore, *T. forthysia* is a member of the red complex species, known to be associated with periodontitis severity in humans [4, 9], while *C. rectus* is also associated with the severity of periodontitis in humans [10, 11]. Thus, the presence of these three species indicated a poor periodontal condition. The total numbers of the 11 periodontitis-related species (mean ± standard error) in the middle-aged and elderly groups were 3.00 ± 0.12 and 3.40 ± 0.11, respectively, which were significantly greater than in the young group (2.10 ± 0.22) when analyzed using Bonferroni's method after analysis of variance (ANOVA) ($P<0.001$).

This is the first known study to analyze periodontal conditions and the presence of periodontitis-related species in Japanese dogs with a focus on age. It is generally accepted that age is an important factor for the development of periodontitis in humans [8]. The present findings demonstrate that age is also associated with the periodontal condition as well as the presence of periodontitis-related species in dogs. Unlike humans, prosthetic intervention is difficult in dogs, even if they lose their teeth; thus, periodontitis in these animals should be regarded as a life-threatening disease. Furthermore, periodontitis is thought to be associated with certain systemic diseases in dogs, such as cardiovascular diseases [7]. Therefore, it is important to consider the prevention of periodontitis for not only periodontal but also systemic health. In order to prevent periodontitis, it is important to keep in mind that age is an important factor related to periodontitis in dogs. Professional removal of dental plaque and calculus is important especially for elderly dogs, and thorough brushing instruction to their owners should be performed to prevent the onset of periodontitis.

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