The ocular surface is a nutrient-rich environment hosting a wide range of microorganisms. The conjunctival sac microbiome is predominantly composed of commensal bacteria that contribute to the preservation of normal eye health by performing essential homeostatic functions that regulate and prevent excessive growth of potential pathogens on the ocular surface. This is achieved through competition for space and nutrients, which helps to maintain a balanced microbial environment. Microorganisms from the normal flora can potentially transition into pathogenic forms if there is tissue damage in the cornea or if the host's ability to resist infection is compromised by immunosuppression.1–4 Previous studies1,2,4–6 have determined the bacterial composition of the eyes of normal dogs utilizing the standard bacterial culture methods. Although the surveys were carried out separately in diverse geographic regions, they consistently revealed an abundance of gram-positive bacteria, with Staphylococcus being the most prevalent, whereas the distribution of other bacterial species varied.1,2,4–6 Understanding the typical microbial composition of the canine conjunctiva is valuable for clinicians as it aids in the diagnosis and treatment of ocular diseases.

OBJECTIVE
To evaluate the prevalence of oral bacteria in the conjunctiva of brachycephalic and nonbrachycephalic dogs.

ANIMALS
12 brachycephalic (9.58 ± 3.55 years) and 12 nonbrachycephalic (8.33 ± 4.92 years) dogs without systemic disease, regardless of breed and sex, were included in the study, and half of the dogs in each group had periodontitis.

METHODS
This prospective study investigated clinical data including craniofacial ratio, ophthalmic examination results, and periodontal status of the included dogs. Bacterial samples were collected by swabbing the oral mucosa and conjunctival surfaces. The presence and quantity of bacteria were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, 16S rRNA sequencing analysis, and the 10-fold dilution method. Statistical analyses were performed to assess correlations and factors influencing the presence of oral bacteria in the conjunctiva.

RESULTS
The most common bacteria in the conjunctival flora in both groups were Micrococcus luteus, Corynebacterium spp, and Staphylococcus spp. The prevalence of oral bacteria on the conjunctival surface was 33%, with a significantly higher incidence in brachycephalic dogs (P = .027). Oral bacteria detected in the conjunctiva were predominantly Frederiksenia canicola, Neisseria spp, and Moraxella spp. Multiple regression analysis identified age, craniofacial ratio, and gingival index as factors influencing the presence of oral bacteria in the conjunctival flora.

CLINICAL RELEVANCE
Oral resident bacteria have often been isolated from severe infectious corneal ulcers. This study provided evidence that brachycephalic dogs may require dental prophylaxis to reduce their oral bacterial load and that the association of oral bacteria in ocular diseases should be considered.

Keywords: brachycephalic dog, conjunctival flora, ocular diseases, oral bacteria, periodontitis
The oral cavity is a unique environment that harbors numerous bacterial species, including anaerobic and aerobic bacteria. Bacteria play a pivotal role in the onset of periodontal disease by creating biofilms that adhere to and populate the gingival sulcus and tooth surface. The ensuing immune response against these bacteria can result in the development of periodontitis. Periodontitis is the most frequently encountered oral disease in dogs, with a prevalence of approximately 80% in patients aged over 2 to 3 years. Several reports using next-generation sequencing to analyze oral microbiomes revealed that the distribution of oral bacteria varies depending on the presence of oral disease and the geographic surfaces where sample collection was performed.

Previous studies have investigated the disparities in bacterial populations inhabiting the conjunctiva and oral cavity of dogs. Brachycephalic dogs are known to have relative macroglossia, which may be predisposed to have tongues closer to the eyes than nonbrachycephalic dogs. This study was designed to compare oral bacteria in brachycephalic and non-brachycephalic dogs and their conjunctival flora and to evaluate the factors affecting the presence of oral bacteria in the conjunctiva.

Methods

Animals

This prospective study was conducted from May to September 2023 and involved client-owned healthy dogs that visited the Seoul National University Veterinary Medical Teaching Hospital. This study was approved by the Seoul National University Institutional Animal Care and Use Committee (SNU-230420-7), and prior consent was obtained from all clients. Cranial characteristics were measured by determining the craniofacial ratio (CFR), which was calculated as the ratio of the muzzle length to the cranial length (Figure 1). Cases with a CFR less than or equal to 0.5 were classified as brachycephalic breeds. Diet types that are known to change the oral microbiome were also evaluated. Dogs with a recent history of topical administration of eye drops such as antibiotics, immunosuppressants, and NSAIDS, and those currently prescribed such medications within 1 month of their hospital visit, were excluded from the experimental group due to their potential impact on bacterial populations.

Sampling collection and culture protocol

Samples were obtained from the conjunctiva and the oral cavity. For each dog, a single eye free of eye drops or in direct contact with the tongue was selected. For example, a Miniature Poodle (A) has a CFR of 0.60 (62-mm muzzle length/103-mm cranial length), and a Chihuahua (B) has a CFR of 0.24 (18-mm muzzle length/75-mm cranial length) each.

Figure 1—Craniofacial ratio (CFR) of the dog, which is obtained by dividing the muzzle length by the cranial length. The muzzle length (a to b) is measured from the tip of the nose to the dorsal end of the nasal planum, while the cranial length (b to c) is measured from the occipital protuberance to the tip of the nose. As an example, a Miniature Poodle (A) has a CFR of 0.60 (62-mm muzzle length/103-mm cranial length), and a Chihuahua (B) has a CFR of 0.24 (18-mm muzzle length/75-mm cranial length) each.
were incubated under both aerobic and anaerobic conditions. An anaerobic chamber with a packet (AnaeroGen; Oxoid) was used to cultivate the anaerobic bacteria. After incubation, 3 to 4 putative colonies from each sample were selected and subcultured on blood agar plates to obtain a single colony. Simultaneously, to estimate the number of viable bacteria in the sample, the total CFU per milliliter in conjunctival and oral samples was also calculated using the 10-fold dilution method.19

### Bacterial identification

Aerobic and anaerobic bacterial isolates were identified using both matrix-assisted laser desorption/ionization time-of-flight mass/spectrometry (Microflex LRF; Bruker Daltonics) and 16S rRNA sequencing analysis.20 The viable single colony was applied to matrix-assisted laser desorption/ionization time-of-flight analysis, and the isolates displaying an ID score greater than or equal to 2.0 were determined as correctly identified according to the manufacturer's instructions. The genomic DNA of the isolates was extracted using a commercial kit (NISCROprep Plasmid DNA Miniprep S & V kit; Bionics), as previously described11 and amplified using universal primer sets (27F-AGAGTTTGATCCTGGCTCAG and 1492R-TACGCGTACCTTGTTACGACTT; universal primer; Bioneer). Sequence alignment was performed using the Basic Local Alignment Search Tool from the National Center for Biotechnology Information 16S microbial database.

### Statistical analyses

The correlation between the presence of oral bacteria in the conjunctiva and cranial length or periodontitis was evaluated using the Fisher exact test. Additionally, clinical data, including age, CFU, and dental indices between the brachycephalic and nonbrachycephalic groups, were compared using the Mann-Whitney U test. The observed variables were assessed using Pearson correlation analysis. Factors affecting the presence of oral bacteria in the conjunctiva were evaluated using multiple regression analysis. Statistical analyses were performed using SPSS software (SPSS 26; IBM Corp), and P < .05 was considered statistically significant.

### Results

The study included 2 groups consisting of brachycephalic and nonbrachycephalic dogs (12 each). Six dogs in each group were diagnosed with periodontitis (above periodontitis stage II) with grossly observed clinical attachment loss, gingival recession, and GI greater than or equal to 2, whereas the remaining 6 were not classified as having periodontitis. The most represented breeds were the Pomeranian (n = 4 [16.7%]) and Miniature Poodle (4 [16.7%]), followed by Chihuahua (3 [12.5%]), Shih Tzu (2 [8.3%]), Maltese (2 [8.3%]), Beagle (2 [8.3%]), Welsh Corgi (2 [8.3%]), and others (5 [20.8%]). The study group consisted of castrated males (n = 10), an intact female (1), and spayed females (13). The clinical data for the brachycephalic and nonbrachycephalic groups are shown (Table 1). The CFR of the brachycephalic group averaged 0.33 ± 0.10, while the nonbrachycephalic group had an average CFR of 0.60 ± 0.06 (P < .001). No statistically significant differences in age, the CFUs of conjunctival and oral bacteria, CI, PI, and GI were detected between the 2 groups.

Ophthalmic examination including Schirmer tear test-1 and fluorescence staining revealed no remarkable findings except for 1 dog in the brachycephalic group with stage IV periodontitis, which showed moderate conjunctival hyperemia. Abnormality of eyelid conformation and neuro-ophtalmic examination were not observed. Twenty-two dogs were given dry food, and 2 dogs received wet food. Of 2 dogs receiving wet food, 1 dog was brachycephaly with periodontitis, while the other dog was brachycephaly without periodontitis. No oral bacteria were isolated in the conjunctiva of the 2 dogs fed with wet food.

A total of 16 and 23 different bacterial species were isolated from the conjunctiva and oral cavity of the 24 dogs, respectively (Table 2). Bacteria known as oral resident flora were detected in the conjunctiva of 8 out of 24 dogs.21–26 Of these, 7 (7/12 [58.3%]) belonged to the brachycephalic group and 1 (1/12 [8.3%]) belonged to the nonbrachycephalic group, with a significantly higher incidence of oral bacteria present in the conjunctiva of brachycephalic dogs than in the nonbrachycephalic dogs (P = .027). The

### Table 1—Clinical data for age, craniofacial ratio, CFUs of conjunctiva and oral cavity, and dental indices of the 12 brachycephalic dogs and 12 nonbrachycephalic dogs between May and September 2023.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Brachycephalic group</th>
<th>Nonbrachycephalic group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>9.58 ± 3.55</td>
<td>8.33 ± 4.92</td>
<td>.27</td>
</tr>
<tr>
<td>CFR</td>
<td>0.33 ± 0.10</td>
<td>0.60 ± 0.06</td>
<td>&lt;.001a</td>
</tr>
<tr>
<td>Conjunctival bacterial concentration (log CFU/mL)</td>
<td>3.73 ± 0.71</td>
<td>3.49 ± 0.33</td>
<td>.14</td>
</tr>
<tr>
<td>Oral bacterial concentration (log CFU/mL)</td>
<td>6.65 ± 0.78</td>
<td>6.66 ± 0.61</td>
<td>.93</td>
</tr>
<tr>
<td>CI</td>
<td>1.92 ± 1.00</td>
<td>2.08 ± 0.90</td>
<td>.71</td>
</tr>
<tr>
<td>PI</td>
<td>2.08 ± 1.00</td>
<td>2.17 ± 0.94</td>
<td>.89</td>
</tr>
<tr>
<td>GI</td>
<td>1.67 ± 1.15</td>
<td>1.42 ± 1.16</td>
<td>.63</td>
</tr>
</tbody>
</table>

All patients had no underlying medical conditions that could affect healing and were not receiving any medications. Dental indices (calculus index [CI], gingival index [GI] and plaque index [PI]) were assessed in the order scored as absent (0), mild (1), moderate (2), and severe (3) by an investigator.

CFR = Craniofacial ratio.

aSignificantly different (P < .001).
bacteria cultivated in both the conjunctiva and oral cavity were Micrococcus luteus, Frederiksenia canis, Pasteurella canis, Moraxella spp, Actinomyces spp, Staphylococcus epidermidis, Staphylococcus sciuri, Neisseria zoodegmatis, Neisseria canis, and Corynebacterium in descending order. The bacterial species cultivated in the conjunctiva and oral cavity of brachycephalic and nonbrachycephalic dogs are listed in Supplementary Table S1.

The number of cases of the same bacterial species isolated from both conjunctiva and oral cavity in the same dog was observed in 8 brachycephalic dogs (8/12 [66.7%]) and 5 nonbrachycephalic dogs (5/12 [41.7%]). Among them, oral resident bacteria were detected only in the conjunctiva of 4 brachycephalic dogs (4/12 [33.3%]), excluding M luteus and Corynebacterium spp, which are known to be the resident flora of the conjunctiva. The bacteria detected in their conjunctiva were Actinomyces spp, F canicola, Moraxella spp, N canis, N zoodegmatis, and P canis, which are known as all members of the oral resident flora. The presence of oral bacteria in the conjunctiva of patients with periodontitis (above periodontitis stage II) was higher (6/12 [50.0%]) than that in the group without periodontitis (2/12 [16.7%]); however, the difference was not significant (P = .19).

The clinical data of the experimental group previously described (Table 1) were evaluated using multiple regression analysis (Table 4). Among the various factors, age (P = .029), CFR (P = .004), and GI (P = .001) were significantly associated with the presence of bacteria known as the oral resident flora in the conjunctiva. Logarithmic ocular bacterial CFUs (P = .77), logarithmic oral bacterial CFUs (P = .33), CI (P = .47), and PI (P = .76) were not significantly correlated.
Discussion

In this study, 18 distinct bacterial species were identified in the conjunctiva of 24 dogs, of which *M. luteus* was the most common, present in 18/24 (75.0%) dogs. *Micrococcus luteus* is a nonpathogenic microorganism commonly found on various inanimate surfaces, soil, and skin surfaces but is known to be present in the normal conjunctival flora of dogs.2,27 The second most identified bacterial genus was *Corynebacterium* (12/24 [50%]) and the most isolated species was *Corynebacterium mastitidis*, which has been identified in the eyes of humans and mice but has not been previously reported in dogs.28,29 The third most frequently isolated bacterial genus was *Staphylococcus* (11/24 [45.8%]), and the most frequently identified species were *Staphylococcus pseudointermedius*, *Staphylococcus intermedius*, and *Staphylococcus delphini*. A previous study30 classified these 3 subspecies together into the *S. intermedius* group. These findings were similar to those of previous studies1,2,4-6 in the field of veterinary ophthalmology.1,2,4-6

Bacteria known as oral resident flora were observed in the conjunctiva of 8 dogs, corresponding to 33.3% (8/24) of all dogs, and the presence of oral bacteria was significantly higher in brachycephalic dogs (*n* = 7) than in nonbrachycephalic dogs (*n* = 1; *P* = .027). This observation may be linked to anatomical or morphological characteristics specific to brachycephalic dogs. It is important to note that brachycephalic dogs exhibit comparatively shorter cranial lengths, which could lead to unintentional contact of their tongues with the eyeballs during typical nose-licking behavior. Brachycephalic dogs possess greater tongue volume than mesencephalic dogs in relation to their cranial length and often display notably larger palpebral fissures than other breeds, which is often associated with shallow orbits, resulting in a condition in which the eyes protrude abnormally and are at an increased risk of injury from external factors.15,16 These features may contribute

### Table 3—Correlation between craniofacial ratio (CFR), dental indices, CFUs, and the 3 most common bacterial species isolated in the conjunctiva and oral cavity were assessed using the Pearson correlation matrix.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Periodontitis</th>
<th>CFUs of conjunctival flora</th>
<th>CFUs of oral flora</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CFR</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Presence</td>
<td>.084</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3. CI</td>
<td>.153</td>
<td>.913&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td>4. PI</td>
<td>.161</td>
<td>.944&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.935&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5. GI</td>
<td>.04</td>
<td>.858&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.817&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CFU of conjunctival flora</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Total</td>
<td>-.254</td>
<td>-.007</td>
<td>-.15</td>
</tr>
<tr>
<td>7. <em>Staphylococcus</em> spp</td>
<td>-.365</td>
<td>-.242</td>
<td>-.265</td>
</tr>
<tr>
<td>8. <em>Corynebacterium</em> spp</td>
<td>-.204</td>
<td>-.171</td>
<td>-.109</td>
</tr>
<tr>
<td>9. <em>Micrococcus</em> spp</td>
<td>.470&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.136</td>
<td>.225</td>
</tr>
<tr>
<td>CFU of oral flora</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Total</td>
<td>.172</td>
<td>.460&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.504&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>11. <em>Neisseria</em> spp</td>
<td>.376</td>
<td>.041</td>
<td>.037</td>
</tr>
<tr>
<td>12. <em>Frederiksenia</em> spp</td>
<td>.075</td>
<td>-.202</td>
<td>-.234</td>
</tr>
<tr>
<td>13. <em>Pasteurella</em> spp</td>
<td>-.039</td>
<td>.514&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.546&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The logarithmic CFU was compared with other variables.

CI = Calculus index. GI = Gingival index. PI = Plaque index.

*P* < .05, *P* < .01, significantly different interactions.

### Table 4—The effects of age, craniofacial ratio (CFR), conjunctival and oral CFUs, and dental indices on the presence of oral bacteria in the conjunctiva were assessed using multiple regression analysis (backward mode).

<table>
<thead>
<tr>
<th>Variables</th>
<th>β</th>
<th>SE</th>
<th>95% Confidence interval</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.019</td>
<td>0.287</td>
<td>0.420 to 1.617</td>
<td>.002&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age (y)</td>
<td>-0.046</td>
<td>0.020</td>
<td>-0.087 to -0.005</td>
<td>.029&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CFR</td>
<td>-1.496</td>
<td>0.464</td>
<td>-2.463 to -0.529</td>
<td>.004&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Conjunctival bacterial concentration (log CFU/mL)</td>
<td>0.114</td>
<td>0.113</td>
<td>-0.203 to 0.430</td>
<td>.77</td>
</tr>
<tr>
<td>Oral bacterial concentration (log CFU/mL)</td>
<td>-0.123</td>
<td>0.150</td>
<td>-0.379 to 0.133</td>
<td>.33</td>
</tr>
<tr>
<td>CI</td>
<td>0.143</td>
<td>0.155</td>
<td>-0.183 to 0.469</td>
<td>.47</td>
</tr>
<tr>
<td>PI</td>
<td>-0.012</td>
<td>0.271</td>
<td>-0.587 to 0.563</td>
<td>.76</td>
</tr>
<tr>
<td>GI</td>
<td>0.277</td>
<td>0.073</td>
<td>0.125 to 0.430</td>
<td>.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Multiple *R*<sup>2</sup> = 0.533; adjusted *R*<sup>2</sup> = 0.463; *F* statistic = 7.603; *P* = .001.

β = Estimated regression coefficient. CI = Calculus index. GI = Gingival index. PI = Plaque index.

*P* < .05, *P* < .01, significant differences.
to the high incidence of oral bacteria in the ocular flora. Meanwhile, diets and dental fractures with pulp exposure may affect oral microbial composition. A previous study\textsuperscript{31} reported that dry food feeding was associated with less malodor and dental plaque, and a healthier oral microbiota compared to wet food feeding. Two brachycephalic dogs out of a total of 24 dogs were fed wet food in this study. No oral bacteria were isolated in the conjunctiva of these dogs. Another study\textsuperscript{32} confirmed the oral microbiome with endodontic infection was more plentiful than without endodontic infection. Also, rotation of teeth with a lack of spacing may play a role in the early onset of periodontitis.\textsuperscript{33} In this study, no crown fractures were identified in the teeth sampled and rotated teeth were generally considered in brachycephalic dogs.

In the 7 brachycephalic and 1 nonbrachycephalic dogs, the most prevalent oral bacteria identified in the conjunctiva was \textit{F. canincola}, which accounted for the largest proportion (4/8 [50%]), followed by \textit{Neisseria spp} (3/8 [37.5%]), \textit{Moraxella spp} (3/8 [37.5%]), and \textit{Pasteurella spp} (2/8 [25%]). \textit{Actinomyces spp}, \textit{Streptococcus salivarius}, \textit{Bergeyella zoohelcum}, \textit{Enterococcus faecalis}, and \textit{Kocuria marina} were also documented to be present in the oral cavity and upper respiratory tract.\textsuperscript{34} \textit{Neisseria spp}, \textit{Moraxella spp}, \textit{Pasteurella spp}, \textit{Actinomyces spp}, \textit{S. salivarius}, \textit{Z. zoohelcum}, and \textit{K. marina} have also been documented in the conjunctiva, age and saliva of normal dogs.\textsuperscript{8,21-24,26} \textit{Enterococcus faecalis} have been reported to be present in the oral cavity of dogs with periodontitis.\textsuperscript{34}

In this study, only 4 of 8 dogs in which bacteria known as oral resident flora were detected in the conjunctiva showed the simultaneous presence of the same species in both the conjunctiva and oral cavity. The reason for this discrepancy is likely due to the fact that the experimenter did not isolate all the bacteria in the culture, but rather a few 3 to 4 putative colonies, so the dominant species in the oral cavity would have been different for each subject. Therefore, it is recommended that a microbiome assay be performed in future studies to achieve a complete analysis of the conjunctival and oral microbiota and to overcome this inconsistency.

Several case reports in veterinary medicine have described instances of oral bacteria that were observed in this study in the eyes of dogs with ocular diseases.\textsuperscript{35-38} In a previous study,\textsuperscript{35} \textit{Actinomyces bowdennii} was identified in the corneal stromal abscesses associated with ulcerative keratitis. The bacterium exhibited antibiotic resistance and was refractory to treatment. Consequently, a conjunctival graft was performed with superficial keratotomy.\textsuperscript{35} Another study reported the isolation of \textit{Moraxella canis} from a bulldog with a corneal ulcer accompanied by conjunctival hyperemia. Because the depth of the ulcer was greater than 70% of the central corneal thickness, a conjunctival graft was performed.\textsuperscript{36} The other study\textsuperscript{37} documented the effectiveness of 2 corneal cross-linking protocols in dogs with infectious keratitis. \textit{Pasteurella dagmatis} and \textit{F. canincola} were each identified in 1 dog with infectious keratitis. One case report\textsuperscript{39} described a nonhealing corneal ulcer with the coinfection of \textit{E. faecalis} and \textit{Curvularia spp} in a Bichon Frise dog. These reports suggest that the oral bacteria found in the conjunctiva of the 8 dogs in this study could be associated with the ecological balance of the typical ocular flora. In addition, aggressive therapies such as conjunctival grafts, corneal crosslinking protocols, or the use of next-generation antibiotics could be considered for ocular disorders associated with oral bacterial infections.

All dogs with oral resident flora identified in the conjunctiva exhibited no remarkable findings except mild hyperemia in 1 dog, but none were diagnosed with ocular surface disease. Previous studies have shown that oral bacteria can contribute to corneal diseases, underscoring the importance of proactive measures to prevent such occurrences. It is possible to completely eliminate contact between oral bacteria and the conjunctiva in brachycephalic dogs because the anatomical features of the tongue are more likely to come into contact with the eyes. Therefore, regular dental exams may be essential to evaluate the existence of oral disorders such as periodontitis, which could potentially contribute to an elevated bacterial load.\textsuperscript{15,16,33} Early interventions such as regular oral home care and professional dental cleaning can be initiated if dental issues are identified. In addition, it is advisable for brachycephalic dogs to use a corneal shield during dental cleaning procedures to prevent corneal dryness due to anesthesia and safeguard the eye from potential exposure to oral bacteria.

As a result of multiple regression analysis to evaluate factors affecting the detection of oral bacteria in the conjunctiva, age ($P = .029$), CFR ($P = .004$), and GI ($P = .001$) were found to be significant variables. As certain dogs could not undergo general anesthesia or sedation, dental indices (CI, PI, and GI) were conducted to ascertain the probability of periodontitis through oral examination. CFR was identified as the most influential variable with the unstandardized coefficients of $-1.496$.

Age and GI also had significant impacts on the identification of oral bacteria in the conjunctiva, but their effects were relatively small. It is speculated that the aging process is accompanied by a decline in the immune system, potentially resulting in an augmentation of oral flora, that might be more vulnerable to infections. Although GI was proportional to the severity of periodontitis, it was unable to clarify causality. In addition, because sample collecting was performed as 1 sample per tooth at a single time point, bacteria that could not be cultivated were beyond the scope of this study. Also, diets and systemic conditions at the time of sampling may have affected the results. Moreover, even when the same bacteria were identified in both the conjunctiva and oral cavity, the genetic homogeneity of the same bacteria from the 2 locations could not be confirmed. Finally, because sedation or general anesthesia could not be performed, periodontitis indices could not be assessed for all patients, which prevented us from...
distinguishing precise periodontitis stages, including the presence or absence of oral bacteria based on stage. In future studies, expanding the number of experimental groups could potentially offer a more comprehensive understanding of these variables and enhance confidence in identifying the frequency of bacterial presence within the normal conjunctival flora. Furthermore, it will be essential to employ microbiome analysis and molecular characterization by utilizing multilocus sequence typing or whole-genome sequencing to identify genetic homogeneity. This approach would enable the investigation of potential associations between the same bacteria identified in both locations, providing a more in-depth understanding of the ocular microbiota.

This preliminary study showed that the incidence of oral bacteria in the conjunctiva is significantly higher in brachycephalic patients than in nonbrachycephalic patients. Additionally, factors that could affect the presence of oral bacteria in the conjunctiva include CFR, age, and degree of gingival inflammation. This underscores the need for the early treatment and prevention of periodontitis, especially in brachycephalic dogs, as it can potentially cause ocular disease or adversely affect eye health.

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References


Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org