Regular Articles

Identification of *Helicobacter* and *Wolinella* spp. in Oral Cavity of Toy Breed Dogs With Periodontal Disease

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**Introduction**

Periodontal diseases including gingivitis and periodontitis are the most common oral cavity infectious and inflammatory diseases in dogs older than 2 years of age. 

Periodontal diseases could progressively destroy the supporting structures of the teeth, resulting in early tooth loss in dogs. 

Plaque and calculus are the crucial place for bacterial colonization and spreading in oral cavity and gastrointestinal tract of small animals. Although gastric infection due to *Helicobacter pylori* is a major health problem in humans, the significance of *H. pylori* in dogs and any correlation to gastric dysfunctions is still largely unclear. However, different species of *Helicobacter* genus including *Helicobacter heilmannii*, *Helicobacter felis*, *Helicobacter bizzozeronii*, *Helicobacter salomonis*, *Helicobacter bilis*, and *Helicobacter cyanogastricus* have been detected in gastrointestinal tract of dogs. 

*Helicobacter* in dogs could be transferred to humans, pointing the importance of *Helicobacter* detection and treatment in accompanying animals. It has been shown that *Wolinella* spp. rather than *Helicobacter* spp. are the predominant *Helicobacteraceae* in the oral cavity of dogs. 

However, the correlation of *Helicobacteraceae* infection in canine oral cavity with periodontal diseases was poorly understood. Regarding to importance of oral cavity health in dogs and the owners, the present study was designed to identify the infectious rate of *Helicobacter* spp. and *Wolinella* in oral cavity of dogs with periodontal diseases. 

**Materials and Methods**

**Animals and Sampling Procedures**

Sixty-two client-owned toy breed dogs (Dachshund, Maltese, Terrier, Pekingese, Pomeranian, Pug, and Shih Tzu), with age > 6 years (average 9 years) from both sexes with no history of active gastritis were evaluated in this study. Most of the dogs have not been receiving proper dental health care such as tooth brushing or dental cleaning, and only some of them were using a dental food or chew. Thirty-one animals (17 males and 14 females) were assigned to periodontitis group when the oral cavity infection was confirmed with clinical examinations by board-certified small animal internist. The rest of the dogs were categorized as a healthy control (15 males and 16 females) when there was no sign of periodontitis with clinical examinations. Each tooth was evaluated around its entire circumference at a minimum of 8 locations. The presence of periodontal disease was confirmed in all suspected...
DNA was extracted from mix of saliva and dental plaque samples using DNeasy tissue kit (Qiagen, Hilden, Germany) according to the manufacturer’s instruction. Polymerase chain reaction (PCR) amplification was performed in a final volume of 25 µL containing 3 µL of extracted DNA, 2 µL of ×10 PCR buffer (Fermentas, Lithuania), 0.5 µL of dNTP, 0.75 µL MgCl2, 1 µL primer, 0.25 U of Taq DNA polymerase (Fermentas, Lithuania), and 17.5 µL of deH2O. Primer sequences and PCR conditions are presented in Table 1. The resulting PCR products were developed on electrophoresis gel (1.5% [w/v] with 0.3% ethidium bromide in 10% tris-borate ethylenediaminetetraacetic acid buffer) and were visualized under ultraviolet transilluminator. Size of the expected fragments was compared with a 100 bp reference marker (Fig 1).

**Sequencing**

DNA sequences were analyzed by BioEdit software. Consensus sequences were obtained with identification of the partial 16S rRNA gene sequences and verified by comparison of the consensus sequences to the National Center for Biotechnology Information database through the algorithm Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST).

**Construction of Phylogenetic Tree**

Selected, aligned, and edited sequences of *H. felis* 16S rRNA directed to phylogenetic tree by using MEGA 5.3 software package.

Tree was constructed using neighbor-joining algorithm under the global gap removal option and Kimura’s 2-parameter substitution model. Robustness of phylogenetic analysis was measured by bootstrap analysis with 10,000 replications. Out group was also added to dataset (Fig 2).

**Statistical Analysis**

Data were analyzed using SPSS statistics version 16. Presence of *Helicobacter* and *Wolinella* spp. in oral cavity was assessed using Fisher’s exact test. Fisher’s exact test was also used for the association of assessment between the presence of *Helicobacter* and *Wolinella* genus with periodontal disease. Chi-square test was used for comparison of the frequencies. The threshold of significance was set at \( P < 0.05 \).

**Results**

**Detection of *Helicobacter* and *Wolinella* spp. in Oral Cavity of Experimental Groups**

*Helicobacter* spp. were detected in 26 of 31 (83.8%) and 16 of 31 (51.6%) oral samples in periodontitis and healthy groups, respectively. Our results showed significant correlation between periodontitis in dogs and *Helicobacter* spp. infection (\( P = .007 \)). *Helicobacter* heilmannii was detected in 10 of 31 (32.2%) periodontitis and 9 of 31 (29%) healthy dogs. *H. felis* was only detected in 3 of 31 (9.6%) periodontitis group and showed significant correlation with periodontitis (\( P < .001 \)). *Helicobacter pylori* was not detected in any sample. *Wolinella* was identified in 28 of 31 (90.3%) and 14 of 31 (45.1%) periodontitis and healthy groups, respectively. Our results showed a significant correlation between periodontitis in dogs and *Wolinella* infection (\( P = .002 \)) (Table 2).

**BLAST Result**

BLAST result of 3 *H. felis* samples (P8, P14, P15) revealed that closest *UreasB* gene hits in National Center for Biotechnology Information were Pakistan (AC: JF804945, etc.), UK (FQ670179), and France (X690980) with 99% similarity. Also, BLAST results of 2 sequenced samples of *Wolinella* showed 93% similarity to Iran (JN869512), USA (HM277577, HM272351), and some other databases.

### Table 1

<table>
<thead>
<tr>
<th>Target Genes</th>
<th>Reference</th>
<th>Primer Sequence (5’→3’)</th>
<th>PCR Fragment (bp)</th>
<th>PCR Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA genes of <em>Wolinella</em> spp.</td>
<td>[9]</td>
<td>(WOL1, F): AAA GAG CAC GTA GGC GGC</td>
<td>440</td>
<td>94°C for 4 min; 34 cycles for 50 s; 72°C for 4 min</td>
</tr>
<tr>
<td>16S rRNA genes of <em>Helicobacter</em> spp.</td>
<td>[9]</td>
<td>(WOL2, R): CCC GAA CTG TAA CTA TCT TAG AC</td>
<td>200</td>
<td>94°C for 4 min; 34 cycles for 40 s; 72°C for 4 min</td>
</tr>
<tr>
<td><em>ureC</em> gene (<em>H. pylori</em>)</td>
<td>[10]</td>
<td>(F): GGA TAA CCT TTT AGG GGT GTT AGG GG</td>
<td>294</td>
<td>94°C for 4 min; 34 cycles for 50 s; 72°C for 4 min</td>
</tr>
<tr>
<td><em>ureB</em> gene (<em>H. heilmannii</em>)</td>
<td>[11]</td>
<td>(F): GGG CGA TAA AGT GGC CTG G</td>
<td>580</td>
<td>94°C for 4 min; 34 cycles for 40 s; 72°C for 4 min</td>
</tr>
<tr>
<td><em>ureA</em> and <em>ureB</em> genes (<em>H. felis</em>)</td>
<td>[12]</td>
<td>(F): GTG AAG CGA CTA AAG ATA AAC AAT (R): GCA CCA AAT CTA ATT CAT AAC AGG</td>
<td>241</td>
<td>94°C for 4 min; 36 cycles for 40 s; 72°C for 4 min</td>
</tr>
</tbody>
</table>

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Fig. 1. Genus and strain-specific PCR. (A) Helicobacter genus (1200 bp), (B) Wolinella genus (440 bp), (C) H. heilmannii (580 bp), and (D) H. felis (241 bp).

Fig. 2. Phylogenetic tree constructed based on partial nucleotide sequences of urease gene for Helicobacter spp. with MEGA 5.3. Sequenced samples in this study marked by Brucella suis UreA were used as out group in the tree. The tree was constructed by using the neighbor-joining (NJ) algorithm. Units at the bottom of the tree indicate the number of substitution events. The dataset was resampled 10,000 times using the bootstrap method. The relationship of Helicobacter sequences in 16S rDNA reconstructed from the oral cavity with each other and with obtained sequences for a variety of Helicobacteraceae in Gene bank was surveyed further using phylogenetic tree. Sequences of Helicobacteraceae from the oral cavity of 3 dogs were very similar to each other and clustered most closely with H. felis sequences. The other near clusters are H. heilmannii, H. felis, H. salomonis, H. baculiformis, and H. pylori.
Table 2
Prevalence of Helicobacter and Wolinella spp. in Experimental Groups

<table>
<thead>
<tr>
<th>Bacterial Infection</th>
<th>Periodontitis (31) N (%)</th>
<th>Control (31) N (%)</th>
<th>Total (62)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helicobacter spp.</td>
<td>26 (61.9%)</td>
<td>16 (38.1%)</td>
<td>42</td>
</tr>
<tr>
<td>Helicobacter pylori</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Helicobacter heilmannii</td>
<td>10 (52.6%)</td>
<td>9 (47.4%)</td>
<td>19</td>
</tr>
<tr>
<td>Helicobacter felis</td>
<td>3 (100%)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Wolinella spp.</td>
<td>28 (66.7%)</td>
<td>14 (33.3%)</td>
<td>42</td>
</tr>
</tbody>
</table>

* P = .007 compared with control dogs.
** P < .01 compared with control dogs.
*** P = .002 compared with control dogs.

Discussion

Oral cavity infections have been identified as crucial causes not only of systemic infections such as cardiovascular, gastrointestinal, and respiratory diseases but also of local lesions such as gingivitis and periodontitis.13-16 The dental plaque was identified as the main infection reservoir in the oral cavity for Wolinella succinogenes, Arcobacter butzleri, Helicobacter spp., Campylobacter, and Arcobacter in human and domestic pets.17 Based on the results of our study, Helicobacteraceae infections are relevant periodontal infection in pet dogs. In our study, H. heilmannii and H. felis were the main oral cavity infections in toy breed pet dogs with periodontitis. Our results are in line with a study that found the same trend for prevalence of H. felis and H. heilmannii in the oral cavity of stray dogs.18 In our study, H. pylori was not detected in the oral cavity of pet dogs. H. pylori was not detected in oral cavity and stomach of stray dogs.19 We identified that, among Helicobacteraceae family, Wolinella had the highest bacterial infection rate in dogs with periodontitis. Craven et al.20 previously showed that Wolinella spp. is the most common member of the Helicobacteraceae family in the oral cavity of dogs. BLAST results revealed that primers from the study by Craven et al.20 have been false-positive with Desulfomicrobiurnorale. Review of literature indicated that Desulfomicrobiurnorale could be involved in bacterial infection of dogs with periodontitis. Furthermore, Desulfomicrobiurnorale was also isolated from human patients with periodontitis.21 Nonpylori Helicobacter spp. detected in the oral cavity of dogs may act as a source of Helicobacter spp. infection which could be transferred to humans mainly through oral-to-oral transmission.22 Approximately, 0.25%-1.7% of human patients suffering from gastric disorders has been diagnosed with gastric Helicobacter spp. of pet dogs.23 Wolinella transfer from dogs to human has not been identified until now.

Conclusion

In this study, we identified a higher prevalence of Helicobacter and Wolinella spp. bacterial infections in dogs with periodontitis. Surprisingly, Wolinella spp. rather than Helicobacter spp. was the most frequently detected Helicobacteriaceae in the oral cavity of dogs with periodontal diseases. Furthermore, we showed in our study that Helicobacter and Wolinella spp. are correlated with periodontal disease in dogs. The oral cavity of dogs with periodontitis should be considered as a location of Helicobacteriaceae colonization. Although the mode of acquisition of Helicobacteriaceae by human has not been determined, it has been suggested that licking by a pet could be a possible transmission route. Further investigation in this area is required to study the risk posed by contact with the oral cavity of dogs with periodontitis.

References