

Comparative molecular detection of *Helicobacter* spp. and *Wolinella* in the oral cavity of cats and dogs with periodontal disease and healthy individuals

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ABSTRACT

Background and Objectives: *Helicobacter* and *Wolinella* are gram-negative bacteria belonging to the *Helicobacteraceae* family. While *Helicobacter* species are well-known for their role in gastric disorders, emerging evidence suggests their presence in the oral cavity and potential involvement in periodontal diseases.

Materials and Methods: *Helicobacter* and *Wolinella* species were investigated in 122 saliva and periodontal plaque samples from dogs and cats by DNA extraction, PCR amplification, and 16S rDNA gene identification.

Results: Comparing the periodontitis group and the healthy group, a higher incidence of positive *Wolinella* and *Helicobacter* species was shown in both dog and cat groups. 16S rDNA genes of *Helicobacter* were detected in 60% of the cats and 67.7% of the dogs. Detection of 16S rDNA genes of the *Wolinella* group in felines was 78.3% which was higher than in canines (67.7%). *Helicobacter felis* (35%) was the most common species detected in cats, contrary to dogs, in which *Helicobacter heilmannii* (30%) detection was higher (in both groups). *Helicobacter pylori* was not detected in either group.

Conclusion: Comparing the occurrence of *Helicobacter* and *Wolinella* in the mouths of dogs and cats, their association with periodontal disease, and the possibility of a common source of infections between humans and companion animals is of great importance for the management of oral health in animals and humans.

Keywords: *Helicobacter*; *Wolinella*; Cats; Dogs; Saliva; Periodontitis; Polymerase chain reaction

INTRODUCTION

Helicobacter and *Wolinella* are gram-negative bacteria that belong to the *Helicobacteraceae* family. *Helicobacter*, a genus of bacteria, holds significant importance in both human and animal gastrointestinal tracts. Apart from *Helicobacter pylori*, which is known to cause infection, other spiral bacteria found in the digestive tracts of humans, dogs, and cats have

also been identified as potential sources of common zoonotic infections in humans and companion animals. Many non-*H. pylori* *Helicobacters* are capable of inducing gastric disorders in humans, such as gastritis, gastric ulceration, and mucosa-associated lymphoid tissue (MALT) abnormalities. Notable examples include *H. pylori*, *Helicobacter heilmannii*, and *Helicobacter felis*, which are commonly associated with human gastric illnesses (1-3). *Wolinella*, on

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the other hand, has been less extensively studied but shares specific characteristics with *Helicobacter* and may play a role in oral health dynamics.

In the feline stomach, *H. heilmannii* is the most common gastric *Helicobacter*, frequently found together with species like *H. salomonis* or *H. felis*. These cannot be distinguished from each other using routine light microscopy (4).

Interestingly, the first *Helicobacter* isolated from a dog's stomach was *H. felis*, characterized by its large, spiral morphology and the presence of 10 to 17 polar flagella (5).

The *Wolinella* genus is a member of the *Proteobacteria* epsilon subclass, and it was first discovered in the rumen of cattle. *Wolinella succinogenes* was initially discovered in the oral chamber of dogs and was thought to be non-pathogenic at the time. However, analysis of the *Wolinella succinogenes* genome revealed the presence of similar genes in common with *H. pylori* and *Campylobacter jejuni* that are pathogenic. As a result, its non-pathogenicity was questioned, and further research was needed (6).

Periodontal diseases, which affect the gingiva, teeth, and surrounding structures, are prevalent oral health conditions in dogs and cats. While *Helicobacter* research focuses primarily on its role in gastric disorders, emerging evidence suggests its presence in the oral cavity and potential involvement in periodontal diseases. Understanding the comparative aspects of *Helicobacter* in the mouths of dogs and cats and their potential associations with periodontal diseases is crucial for comprehensive oral health management in these animals.

Investigations into the prevalence of *Helicobacter* species in the oral cavity of dogs and cats have provided intriguing insights. Studies by Smith et al. have identified the presence of *Helicobacter* species, including *H. pylori*, *H. heilmannii*, and *H. felis*, in the oral chambers of dogs and cats. These findings indicate that *Helicobacter* colonization extends beyond the gastrointestinal tract, suggesting potential implications for oral health (7).

Helicobacter species exhibit various characteristics that may contribute to the pathogenesis of periodontal diseases. One notable aspect is their ability to produce virulence factors, such as urease and proteases, which can impact the oral environment. Urease, an enzyme produced by *Helicobacter*, hydrolyzes urea to ammonia, leading to an alkaline pH that may disrupt the balance of the oral microbiota and

contribute to tissue damage. Also, *Helicobacter*'s proteases can degrade host proteins and exacerbate inflammation and tissue destruction in the periodontium.

Although research on *Wolinella* in the context of oral health is scarce, its genetic and phenotypic similarities to *Helicobacter* warrant attention. *Wolinella* species have been primarily studied for gastrointestinal and periodontal diseases in humans. In this regard, several cases have been discovered in which *Wolinella* is the causative agent, including: An uncultured *Wolinella* organism called *Candidatus Wolinella africanus* has been found in humans with esophageal squamous cell carcinoma. The same organism has been found in humans living in Venezuela who had a combined disease of the stomach, esophagus, and oral cavity, but were not clinically symptomatic. In animals, the organism has also been found in the stomach of a horse with a gastric ulcer. In addition, a new species of *Wolinella* has been identified in the digestive tract of a sea lion with gastritis (6, 8).

The adaptation of *Helicobacter* spp. to different environments within the gastrointestinal tract suggests that *Wolinella* spp. might be better suited for colonizing the squamous epithelium due to the similarity with the lining of the canine oral cavity and the rumen and esophagus (6).

Comparison and research on *Helicobacter* and *Wolinella* in the oral microbiota of dogs and cats represents a significant area of research with potential implications for periodontal disease pathogenesis. The prevalence of these bacterial genera in the oral cavity and their possible associations with periodontal diseases highlight the need for further exploration. Understanding the role of these bacteria in oral health disorders can pave the way for innovative preventive and therapeutic interventions, ultimately promoting improved periodontal health in our beloved companions. Thus, to achieve these goals, this study attempted to provide a detailed comparison between *Helicobacter* and *Wolinella* in the oral cavity of periodontitis and healthy dogs and cats to assist in future research.

MATERIALS AND METHODS

Sample collection. In this study, a total of 122 client-owned adult cats and dogs referred to Small Animal Hospital, Faculty of Veterinary Medicine, Uni-

versity of Tehran (FVM-UT), including 62 healthy (30 cats, 32 dogs) and 62 with periodontal disease (30 cats, 32 dogs), were investigated. Animals having a history of halitosis and a tendency to use wet food in addition to dental calculus or evidence of teeth loss and gingivostomatitis in the clinical examination were considered suspicious of periodontal disease and were confirmed by the increase in the depth of the periodontal pocket (more than 3 mm in dogs and more than 1 mm in cats) in examination with a periodontal probe under light anesthesia.

Animals with no signs of periodontal disease in their history and clinical examinations were classified in the healthy group. Full consent for sampling was obtained from all owners before the sampling process began.

Saliva and other oral secretions were collected using sterile swabs. Dental plaque samples from above and below the gingiva were also collected using hand-holding pincers. These dental plaque samples were removed from the outer surface of all teeth. The specimens were collected into microtubes containing 1cc of Phosphate Buffered Saline (PBS) under strict aseptic conditions, kept at -20°C, and sent to the University of Tehran's Microbiology Laboratory to perform molecular tests.

DNA extraction. The DNA from the oral secretion sample was extracted using a DNA extraction and purification kit (Qiagen, Germany). Initially, 200 microliters of each sample were placed into 200 microliters of AL buffer (Lysis buffer) and 20 microliters of proteinase enzyme were added. The resulting mixture was then homogenized by vortexing, and then heated to 56°C for 10 minutes. DNA was then extracted and purified from all samples according to the kit manufacturer's instructions. To verify the correctness of genome purification, DNA concentration was measured using NanoDrop™ (Thermo Scientific, Wilmington, DE), and 5 µl of extracted DNA was analyzed by electrophoresis on a 1% agarose gel.

PCR amplification and DNA sequencing. To identify *Helicobacter* and *Wolinella* species, 16S rDNA gene identification has been carried out. In contrast, the urease gene has the ability to identify specific *Helicobacter* species such as *H. pylori*, *H. felis*, and *H. heilmannii*. PCR amplification was performed with 16 µl of PCR Master Mix 2x (80Test/25ul-MM2011, Sinaclon, Iran), 1 µl of each primer (Table 1), and 2

µl of template DNA. The final reaction volume in this model was 20 µl. The PCR of this study was performed using an MJ Mini™ thermocycler (Bio-Rad, USA) with initial denaturation at 94°C for 4 min followed by a cycle consisting of a denaturation step at 94°C for 50 s, an annealing step at 50-58°C for 50 s, and an elongation step at 72°C for 60 s. Finally, the final elongation step was performed at 72°C for 4 minutes, and all PCR products in this study were placed on a 1.5% agarose gel for electrophoresis. A gel documentation system was also used to record the images.

Statistical analysis. SPSS statistical software version 27.0 (United States of America) was used for statistical analysis of the data of this study. Also, the prevalence of *Helicobacter* and *Wolinella* species was evaluated using Pearson's two-tailed Chi-Square test. The level of statistical significance of the data was considered $p < 0.05$.

RESULTS

***Helicobacter* species identification through PCR analysis.** The prevalence of *Helicobacter* species and *Wolinella* between periodontitis and healthy groups in saliva and dental plaque samples is indicated in Table 2.

Identification of *Helicobacter* genus. In this study, after PCR, the products were electrophoresed on an agarose gel. Out of 60 saliva and dental plaque samples that were collected from cats, the 16S rDNA genes of *Helicobacter* were detected in 36 of the cats. This issue indicated the involvement of 60% of cats in different types of *Helicobacter*. On the other hand, these genes were detected in 42 of 62 of the canine group, indicating 67.7% infection, which is higher than the feline group. Detection of 16S rDNA genes of the *Wolinella* group in the feline group was 47 of 60 (78.3%), which was higher than the canine group with 42 of 62 (67.7%). The periodontitis patient group had a significantly higher prevalence of *Wolinella* and *Helicobacter* species. Positive cases in both feline and canine groups (86.7% (cats) and 66.6% (dogs) for *Wolinella*) and (63.3% (cats) and 61.9% (dogs) for *Helicobacter*) compared to the healthy group (70% (cats) and 33.3% (dogs) for *Wolinella*) and (56.7% (cats) and 38% (dogs) for *Helicobacter*) respectively (Table 2). According to these Tables, *H. felis* (35%)

Table 1. Primers used to identify the genus and species of *Helicobacter* and *Wolinella*

<i>Helicobacter</i> species	Primer	Oligonucleotide sequence (5'-3')	PCR product size (bp)
16s rDNA of <i>Wolinella</i> Spp	WOL1F	F- AAA GAG CAC GTA GGC GGC	440
	WOL2R	R- CCC GAA CTG TAA CTA TCT TAG AC	
16s rDNA of <i>Helicobacter</i> Spp	C97F	F- GCT ATG ACG GGT ATC C	200
	C05R	R- ACT TCA CCC CAG TCG CTG	
<i>ureC</i> gene	HP-FOR	F- GGA TAA GCT TTT AGG GGT GTTAGG GG	294
<i>H. pylori</i>	HP-REV	R- GCT TAC TTT CTA ACA CTA ACG CGC	
<i>ureB</i> gene	H276F	F- GGG CGA TAA AGT GCG CTT G	580
<i>H. heilmannii</i>	H676R	R- CTG GTC AAT GAG AGC AGG	
<i>ureA</i> and <i>ureB</i> gene <i>H. felis</i>	Fe1F	F-GTG AAG CGA CTA AAG ATA AAC AAT	241
	Fe3R	R- GCA CCA AAT CTA ATT CAT AAG AGC	

Table 2. Isolation numbers of *Helicobacter* spp. and *Wolinella* spp. in oral cavity specimens of healthy and periodontitis groups

	Dog			Cat		
	Periodontitis	Healthy	Total	Periodontitis	Healthy	Total
<i>Wolinella</i> SPP.	28 (66.6%)	14 (33.3%)	42 (67.7%)	26 (86.7%)	21 (70%)	47 (78.3%)
<i>Helicobacter</i> SPP.	26 (61.9%)	16 (38%)	42 (67.7%)	19 (63.3%)	17 (56.7%)	36 (60%)
<i>H. heilmanni</i>	10 (52.6%)	9 (47.3%)	19 (30%)	10 (33.3%)	8 (26.7%)	18 (30%)
<i>H. felis</i>	3 (100%)	0 (0%)	3 (4.8%)	10 (33.3%)	11 (36.7%)	21 (35%)
<i>H. pylori</i>	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

is the most common species detected in cats contrary to dogs which *H. heilmanni*'s (30%) detection was higher (in both healthy and periodontitis groups) ($p < 0.05$). *H. pylori* was not found in any of the groups in this study. After analyzing all results with a two-tailed Chi-Square test with SPSS Statistics software version 27.0, no statistically significant difference was seen between feline and canine groups (p -value > 0.05).

DISCUSSION

Many different studies have been conducted to date to investigate and explore the role of *Helicobacter* in human gastrointestinal diseases. Although the pathogenic effects of these organisms in animal stomachs are milder compared to those of *H. pylori* in humans, some of them can still contribute to the development of chronic gastritis (9). The most commonly reported non-*pylori* organisms in humans are *H. heilmannii* and *H. felis*. Studies have found that

these species have been responsible for causing gastritis in 0.25% to 6% of the human population (10). In addition, studies have shown that the likelihood of *H. heilmannii* infection is higher in humans who are in contact with companion animals such as dogs or cats or farm animals such as pigs (11). A study involving 768 humans with *H. pylori*-induced gastritis found that 74.8% of cases had contact with one or more animals, indicating a possible zoonotic transmission route (12). The importance of this issue is growing as recent research by Majeed Ali Fahad in 2024 indicated a connection between *H. pylori* and diabetes and even heart disease in humans (13).

Miller (1891) and Hunter (1900) were the first to propose that oral infections could lead to systemic disease. Recent studies have also shown that inflammation and periodontal infections are associated with problems with the heart, metabolism, nervous system, lungs, and cancer (14, 15). Elderly individuals, immunocompromised patients, and those receiving chemotherapy or radiation therapy are at an

elevated risk of acquiring systemic infections originating from the oral cavity (16).

For more than 80% of geriatric dogs, periodontal disease is the main cause of tooth loss (17). Furthermore, in human populations, approximately 30% of adults suffer from varying degrees of periodontal disease. A study conducted on referred dogs to the veterinary hospital of Tehran University's Faculty of Veterinary Medicine revealed a 12% prevalence rate for periodontal disease (18).

Most of the bacteria in the *Helicobacter* family that can be found in dogs' mouths are *Wolinella* species, rather than *Helicobacter* species. The main reservoir for the infection of *Wolinella succinogenes*, *Arcobacter butzleri*, *Helicobacter* species, *Campylobacter*, and *Arcobacter* in the oral cavities of humans and domestic pets is the dental plaque (19).

Given the growing population of domestic cats and dogs in Iran, as well as the probability that a large number of these animals will encounter close contact with humans, investigating the transmission of these organisms to other animals and the human population is crucial. Meanwhile, although definitive routes of transmission of *Helicobacter* have not been established yet based on existing research and evidence, in all probable routes (including oral-oral, gastric-oral, and fecal-oral transmission), the oral cavity plays an important role (20, 21).

The results obtained from the current investigation demonstrated that the prevalence of *Wolinella* is higher in cats and dogs with periodontitis compared to *Helicobacter*, which is consistent with the findings of previous research conducted by Simpson et al. in 2010 (22). However, the oral population of *Helicobacter* is still of significant abundance, indicating that it can still be considered an important pathogen for transmission to other species, including humans. The higher prevalence of *Wolinella* in the mouth compared to *Helicobacter* species (although not statistically significant) is thought to be related to several factors. These include pH, redox potential, and nutrient availability in the oral microenvironment. Additionally, the growth of a number of specific species may be dependent on the presence or absence of other organisms in the same microenvironment.

Furthermore, the contamination of *Helicobacter* species in patients with periodontal disease was significantly higher than in healthy individuals. The highest level of contamination in the studied dogs

was related to *H. heilmannii*, while in cats it was related to *H. felis*. In line with the present study, the study conducted by Arfaee et al. in 2012 on 48 dogs also indicated a higher level of contamination with *H. heilmannii* compared to *H. felis* (23).

In this research, none of the examined samples were infected with *H. pylori*. Similar to this, a 2009 PCR-based investigation conducted in Korea showed no evidence of *H. pylori*, but *Helicobacter* species—particularly *H. felis*—were common, suggesting adaptation to feline hosts rather than zoonotic transmission (24). A 2023 meta-analysis confirmed that cats are unlikely to be reservoirs for *H. pylori* infection in humans by discovering no significant correlation between having a cat and the infection (25). Systematic reviews instead point out that although non-*H. pylori Helicobacter* species like *H. felis*, *H. salomonis*, and *H. bizzozeronii*, colonize cats, but they seldom cause gastrointestinal diseases in their dedicated hosts and are only sporadically related to infections in humans, such as gastritis or mucosa-associated lymphoid tissue lymphoma (26, 27). The zoonotic risk of gastric *H. pylori*-like organisms and related genera like *Wolinella* remains unresolved and warrants further study (24, 27).

The higher prevalence of *Helicobacter* and the lower prevalence of *Wolinella* in the canine group, compared to the feline group, were not statistically significant (p -value > 0.05); therefore, no conclusion can be made on each animal being the specific reservoir for each species.

CONCLUSION

The increased incidence of diseases caused by bacteria such as *Helicobacter* and *Wolinella* in dogs and cats with periodontal disease may indicate that there is a link between these *Helicobacter* species and oral diseases in companion animals. Therefore, the mouths and oral plaques of dogs and cats with oral disease and periodontitis can be considered a site for the accumulation of *Helicobacter* species.

The abundance of *Helicobacteraceae* population in the studied area highlights its significance as an essential transmission route, particularly for individuals in contact with domestic cats and dogs. Furthermore, the oral cavity has been identified as a potential source of contamination for other parts of the digestive system. The contamination in this

organ could be attributed to either reflux of gastric secretions or environmental factors, but further research is required to establish a clear understanding. The oral cavity is notably contaminated with *Helicobacter* organisms, particularly *Wolinella*, which supports the potential role of animal health and their oral cavity in transmitting *Helicobacter* infections to humans. Given the significance of these findings, a comprehensive investigation is warranted to explore the factors influencing *Helicobacter* and *Wolinella* contamination in the oral cavity and their role in transmitting infections to humans, as well as the importance of veterinary care for dental health in dogs and cats.

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