 doi:10.20944/preprints202209.0283.v1

Article

Identification and Prevalence of *Helicobacter Pylori* Virulence Genes *Baba* and *Caga* in *Wolinella* Isolates from the Oral Cavity of Dogs

Zahra Jahanshiri [1], Bahar Nayeri-Fasaei [2,*], Shahram Jamshidi [3] and Taghi Zahraei-Salehi[2]

[1] Faculty of Veterinary Medicine, Tehran University, Tehran, Iran; Zjahanshiri@ut.ac.ir
[2] Department of Microbiology and Immunology, Faculty of Veterinary Medicine, Tehran University, Tehran, Iran; Nayerib@ut.ac.ir; tsalehi@ut.ac.ir
[3] Department of Clinical Sciences, Faculty of Veterinary Medicine, Tehran University, Tehran, Iran; shjamshidi@ut.ac.ir
* Correspondence: Nayerib@ut.ac.ir; Tel.: +989122770229

Abstract: *Wolinella* spp. and *Helicobacter* spp. have been repeatedly reported in the oral cavity of dogs and are associated with periodontal disease. *Wolinella* strains predominate in the oral cavity of dogs. The only known species of this genus, *Wolinella succinogenes*, was considered non-pathogenic until sequence analysis of its genome revealed homologous genes resembling virulence factors in *Helicobacter pylori*. This has led researchers to question the nonpathogenic status of *W. succinogenes*. The *cagA* and *babA* genes are examples of crucial virulence factors in *H. pylori* pathogenesis; thus, the present study evaluated the prevalence of these genera and assessed the *Wolinella* strain genome in terms of the presence of these virulence factors. Multiple specific PCR tests were performed on oral secretion samples collected from 62 dogs by sterile cytobrush to evaluate the genera, species, and presence of virulence genes. The species-specific *16s rRNA* genes from the *Helicobacter* and *Wolinella* genera were detected in 58.06% and 83.87% of the oral samples, respectively. *H. pylori* were not detected in the specimens. No *cagA* and *babA* genes were detected in the *Wolinella* spp. or non-pylori *Helicobacter* genomes. Our results confirmed that *Wolinella* spp. is the predominant population compared to *Helicobacter* in the oral cavity of dogs. Apparently, the incidence of *Helicobacter* infections is generally associated with non-pylori Helicobacter organisms. Despite the hypothesis of genomic homology between *W. succinogenes* and *H. pylori*, *cagA* and *babA* virulence genes were not identified in any of the oral samples from the dogs.

Keywords: Wolinella; Virulence genes; *Helicobacter pylori*; genomic homology

1. Introduction

Helicobacter organisms colonize the gastrointestinal tract of humans and other mammalians, particularly dogs and cats[1]. Unlike in humans, *Helicobacter pylori* is not the main organism responsible for chronic gastritis in cats and dogs, even though non-pylori Helicobacter types also are associated with the occurrence of gastritis in humans[1]–[3]. Helicobacter spp. have been detected in the oral cavity of dogs and it has been shown that the dental calculi and the oral cavity discharge of dogs are major reservoirs of Helicobacteraceae infections in the gastrointestinal tract[3], [4]. This means that oral secretion is a possible route of transmission and that the identification and treatment of diseased individuals are important from the perspective of public health[5]–[7].

In contrast to medical surveys, most veterinary reports have published negative results regarding the detection of *H. pylori* in the upper digestive tracts of dogs[8]–[11]. Nonetheless, it has been detected in other parts of their gastrointestinal tracts during gastric and colonic biopsies[12], [13]. In general, Helicobacter spp. are more widely distributed in other sections of the alimentary tract than in the canine oral cavity[9], [13].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 September 2022 doi:10.20944/preprints202209.0283.v1

It has been suggested that, in comparison with Helicobacter spp., Wolinella strains are the predominant organisms in the oral cavity of dogs[9], [10]. Sequencing of the *16S rRNA* gene has shown that Helicobacteraceae in the oral cavity of dogs has been most closely identified with the genus Wolinella[8], [9]. Wolinella appears to prefer to colonize in the squamous epithelium of the upper parts of the alimentary tract and has not been detected in gastric biopsies of dogs[8]–[10], [14], [15]. A positive correlation has been observed between the prevalence of Wolinella strains and the development of periodontal disease in dogs[10].

Wolinella was introduced as a nonpathogenic bacterium until the analysis of complete genome sequencing divulged that *Wolinella succinogenes* shares a large number of genes that encode the virulence factors present in *H. pylori*[16]. This announcement led researchers to question the non-pathogenic status of Wolinella and evaluate its nature more precisely. Because of the observed predominance of the Wolinella population over that of Helicobacter spp. in the oral cavity of dogs as well as the high genomic identity between *W. succinogenes* and *H. pylori*, the current study evaluated the prevalence of these genera in the oral cavity of dogs and assessed the genome of Wolinella spp. in terms of the presence of *cagA* and *babA* as crucial virulence genes found in *H. pylori*[17].

2. Materials and Methods

2.1. Animals and sampling procedures

Sixty-two client-owned or stray dogs over the age of 2 years of either sex that were referred to the hospital of the Faculty of Veterinary Medicine at the University of Tehran were evaluated. The samples were collected randomly, regardless of the incidence of known oral diseases, including periodontitis. Most of the dogs had been deprived of adequate oral health care such as regular brushing or periodic scaling.

All sample collection was performed with the full consent of the owners under the supervision of the Iranian Society for the Prevention of Cruelty to Animals (ethics # IR.UT.VETMED.REC.1401.005) and by avoiding any aggressive or cruel methods. A mixture of saliva, biofilm, and oral secretions were collected using a sterile cytobrush from biofilm that had formed on the molar and premolar teeth of both the maxilla and mandible. This mixture of saliva, oral secretions, and dental biofilm were immediately transferred into a tube containing 1 mL of sterile phosphate-buffered saline and then were stored at a −20°C for further investigation.

2.2. DNA extraction and PCR assays

DNA was extracted from a mixture of saliva and dental plaque using a SinaPure DNA tissue kit (Sinaclon; Iran) according to manufacturer instructions. 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 (Sinaclon; Iran), 0.5 µL of dNTP, 0.75 µL of MgCl2, 1 µL of primer, 0.25 U of Taq DNA polymerase (Sinaclon; Iran), and 17.5 µL of dH2O. The 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 an ultraviolet transilluminator. The sizes of the expected fragments were compared with a 100 bp reference marker (Sinaclon; Iran).

Table 1. Oligonucleotide primers and PCR protocols.

Gene name	Primer sequences	TM	Thermocycler protocols (°C, min)	Repeat	Size (bp)	Ref.
16SrRNA	F:	51	Pre-denaturation: 94°C, 10 min	35×	1200	[9]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 September 2022 doi:10.20944/preprints202209.0283.v1

(*Helicobacter* spp.)	GCTATGACGGGTATCC R: ACTTCACCCCAGTCGCTG	58	Denaturation: 94°C, 1 min Annealing: 58°C, 90 s Extension: 72°C, 2 min Final: 72°c, 15 min			
16SrRNA (*Wolinella* spp.)	F: AAAGAGCACGTAGGCGGC R: CAGGATTCTATCAATGTCAAGCCC	58 64	Pre-denaturation: 95°C, 30s Denaturation: 95°C, 15 min. Annealing: 58°C, 30s Extension: 72°C, 45s Final: 72°C, 7 min.	34×	440	[9]
ureC gene (*H. pylori*)	F: GGATAAGCTTTTAGGGGTGTTAGGGG R: GCTTACTTTCTAACACTAACGCGC	68 64	Pre-denaturation: 94°C, 4 min Denaturation: 94°C, 1 min Annealing: 57°C, 1 min Extension: 72°C, 1 min Final: 72°C, 5 min	37×	293	[18]
cagA	F: GATAACAGGCAAGCTTTTGAGG R: CTGCAAAAGATTGTTTGGCAGA	60 58	Denaturation: 95°C, 1 min Annealing: 56°C, 1 min Extension: 72°C, 1 min Final: 72°C, 7 min	37×	300	[19]
babA	F: AATCCAAAAAGGAGAAAAAGTATGAAA R: TGTTAGTGATTTCGGTGTAGGACA	59 62	Pre-denaturation: 95°C, 5 min Denaturation: 92°C, 1 min Annealing: 59°C, 1 min Extension: 72°C, 1 min Final: 72°C, 5 min	34×	832	[20]

2.3. Gene sequencing

Because no positive control was available with which to confirm the detection of the *Wolinella* spp. genome by PCR assay, one positive sample for 16S rRNA specific primers that was purported to be *Wolinella spp.* was randomly subjected to the Sanger sequencing method. The sequences were analyzed in MEGA7 software. The BLAST result of a sequenced sample of *Wolinella* showed 94% similarity to other databases. The 16S rRNA gene has been recorded in the NCBI database under accession number ON845539.

3. Results

The specific *16s rRNA* genes of the genus Helicobacter and Wolinella were detected in 36 out of 62 (58.06%) and 52 out of 62 (83.87%) oral samples from dogs, respectively. Overall, 57 out of the 62 oral secretion samples (91.94%) were considered infected with the two organisms and only 5 (8.06%) were appraised as healthy. Of the 57 cases that were considered infected, 31 (54.39%) showed concurrent infection with both Wolinella and Helicobacter, 5 (8.77%) were infected only with non-pylori Helicobacters, and 21 (36.84%) were infected only with Wolinella. The *ureC* gene of *H. pylori* was not detected in any of the samples. In the results of these samples, despite the genomic similarity between Wolinella and *H. pylori* stated in the reference articles[16], no *cagA* and *babA* genes were detected in the genome sequence of the genus Wolinella or in non-pylori Helicobacter. (Table 2, Figure1, Figure2)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 September 2022 doi:10.20944/preprints202209.0283.v1

Table 2. Prevalence of genes in experimental specimens.

Gene name	Number of detection (%)
16S rRNA(Wolinella spp.)	52 (83.87%)
16S rRNA(Helicobacter spp.)	36 (58.06%)
ureC (*Helicobacter pylori*)	0 (0%)
cagA	0 (0%)
babA	0 (0%)

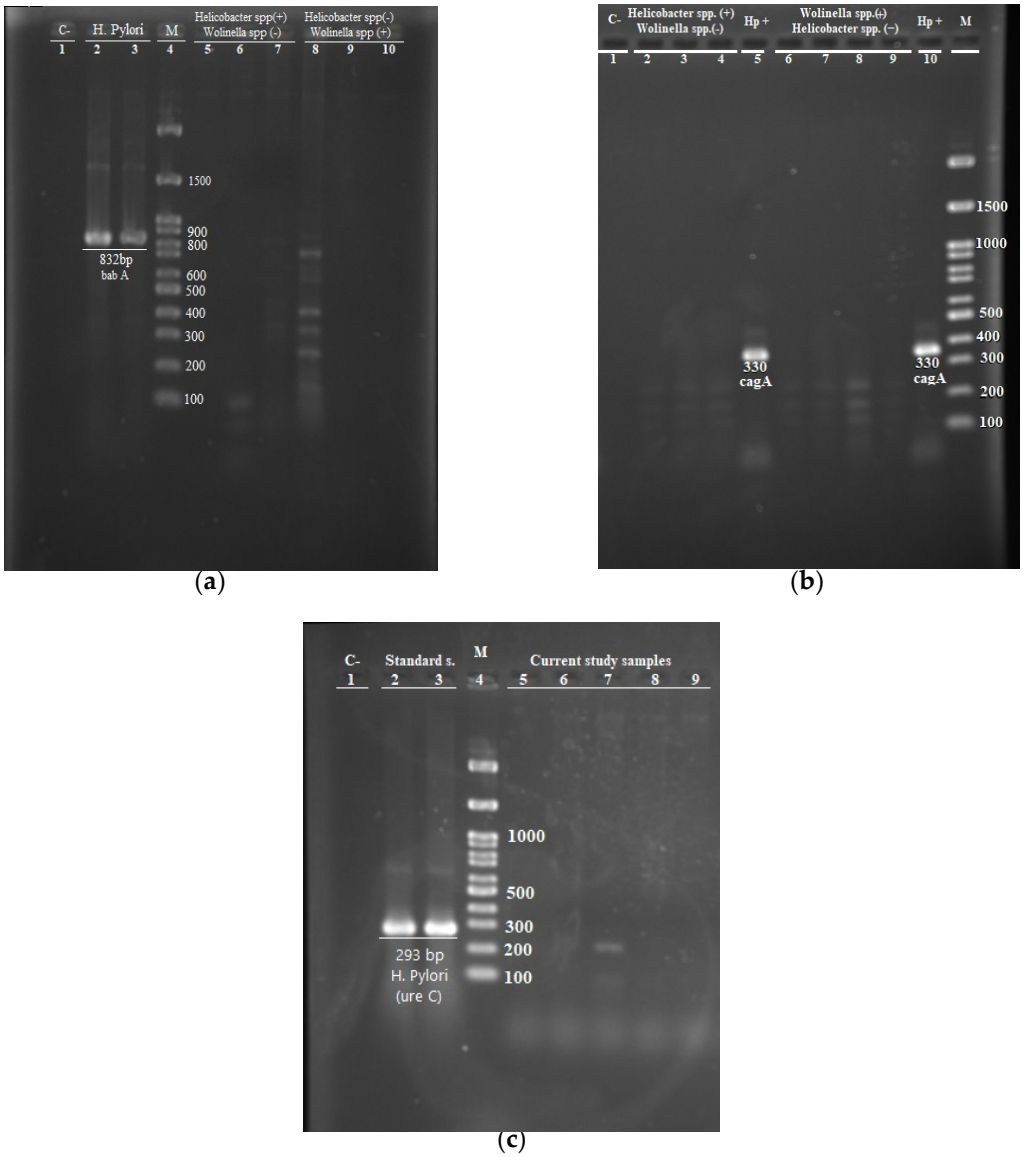
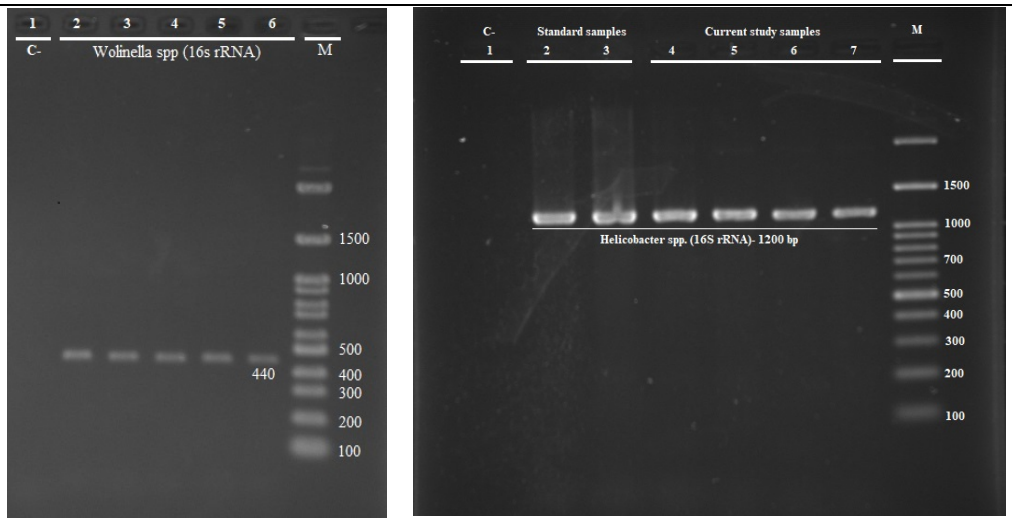


Figure. 1. Genus and species-specific PCR: **(a)** *Wolinella* genus (440 bp): 1 (negative control), 2-6 (study samples), 7 (marker); **(b)** *Helicobacter* genus (1200 bp): 1 (negative control), 2-3 (positive controls), 4-7 (study samples); **(c)** *H. pylori* (293bp): 1 (negative control), 2-3 (positive controls), 4 (marker), 5-9 (study samples).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 September 2022 doi:10.20944/preprints202209.0283.v1



(**a**) (**b**)

Figure. 2. PCR of *H. pylori* virulence genes: **(a)** *babA* (832 bp): 1 (negative control), 2-3 (positive control), 4 (marker), 5-7 (*Helicobacter*-positive samples), 8-10 (*Wolinella*-positive samples); **(b)** *cagA* (330 bp): 1 (negative control) 2-4 (*Helicobacter*-positive samples), 5,10 (positive controls), 6-9 (*Wolinella*-positive samples).

4. Discussion

The resident microbial flora of the canine oral cavity contains a wide variety of aerobic, facultative, and obligate anaerobic organisms.[21] Inflammation and recession in the margins of free gingiva are common findings in small animals.[22] The oral cavity of dogs has been repeatedly reported to be positive for the presence of *Helicobacter* spp.[23] Marshall et al.[24] first reported that the oral cavity is a point of entry for gastric *H. pylori* in humans and the possibility of the development of Helicobacterial gastritis.

Dental plaque and secretions from the oral cavity of dogs play an important role in the conservation of Helicobacteraceae organisms, particularly *Helicobacter* spp. and *Wolinella* strains.[25] Oral cavity discharge from dogs has been suggested as a possible route of transmission of primarily non-pyloric strains of *Helicobacter* to humans.[26] *W. succinogenes*, which was originally isolated from the bovine rumen, also has been detected in the oral cavities of dogs.[27], [28] It seems that the only isolated and cultured species of this genus is *W. succinogenes.*[27]

The prevalence of Helicobacteraceae in the oral cavity may not necessarily be a reflection of the gastric infection load in dogs.[11] The results of the current study indicate that, among Helicobacteraceae members in the oral cavity of dogs, *Wolinella* strains (83.87%) were predominant over *Helicobacter* spp.(58.06%). Note that only the specific set of primers designed by Craven et al.[9] to distinguish *Wolinella* strains from *Helicobacter* species were used in the current study. The results are in accordance with previous studies that have evaluated the prevalence of the same genera in the oral cavity of dogs.[8]–[10]

Furthermore, as evidenced by earlier sequels for inspecting Helicobacteraceae organisms in the gastrointestinal system of dogs, it seems that *Wolinella* spp. are more widely distributed in the oral cavity in comparison with other parts of the alimentary tract.[9] Moreover, there have been several reports of a novel uncultivated species of *Wolinella* known as *Candidatus Wolinella africanus* found in human patients with squamous cell carcinoma of the esophagus in a specimen of a mixture of the stomach, esophagus, and oral cavity of asymptomatic Venezuelans.[14], [29]

The oral cavity of dogs as well as the esophagus and rumen of cattle are lined with squamous epithelia; thus, *Wolinella* may prefer to colonize in this area.[9] However, some reports discuss the detection of a new putative *Wolinella* in gastric biopsies of a sea lion

 doi:10.20944/preprints202209.0283.v1

with gastritis and in thoroughbred horses with gastric ulcers.[30], [31] Nucleotide sequencing and BLAST results have identified *Wolinella* spp. in the dogs of the current study as being more closely associated with *W. succinogenes* than with *Candidatus W. africanus.*

By contrast, published reports state that *H. pylori* was more detectable in other parts of the alimentary tract, including the stomach and colons. Abdi et al.[14] and Torkan et al.[15] detected *H. pylori* in colonic and gastric biopsies of Iranian dogs, respectively. Elhelw et al.[32] performed PCR tests and reported that 62.5% of Egyptian dog stool samples were positive for *H. pylori*. Kubota-Aizawa et al.[33] reported that gastric biopsies collected from two dogs in Japan were infected with the same *H. pylori* strain detected in gastric biopsies from their owners.

H. pylori was not detected in the oral cavity specimens collected in the current study. The helicobacter organisms detected in our specimens were totally associated with non-pyloric species, which is in line with the results of Craven et al.[11] and Nowroozilarki et al.[12] The exact reason why *Wolinella* has a greater propensity to colonize in the squamous epithelium of the oral cavity of dogs instead of *Helicobacter* spp. is not well known. However, it may relate to subtleties in the oral microenvironment, such as pH, reduction-oxidation potential, and nutrient availability. It has been suggested that the presence of specific species such as *Streptococcus mutans* and *Prevotella intermedia* in the bio-film could inhibit the growth of *H. pylori* strains.[34]–[36] It also has been shown that *Porphyromonas* spp and *Fusobacterium nucleatum* can adhere to and trap *H. pylori*.[35]

In addition to its effect on health, the incidence of *Wolinella* spp. has been significantly associated with the development of periodontitis in dogs.[10] This has led researchers to question the nonpathogenic status of *W. succinogenes*. Baar et al.[18] revealed that according to the analysis of the sequence of the *W. succinogenes* (DSM 1740 [ATCC 29543]) 2,110,355bp genome, this bacterium belongs to the epsilon-proteobacteria and is Helicobacteraceae based on 16S rRNA phylogeny. In their comparison of the complete predicted set of *W. succinogenes* proteins with the NCBI database, 32% of pairs were *H. pylori* and 30% of pairs were *C. jejuni*.

The closest hit for 655 proteins were in *H. pylori*.[16] *W. succinogenes* shares numerous homologous genes with its two closest relatives that encode virulence factors (eg, type IV secretory pathway, adhesins, invasins, hemolysins, proteases, and cytotoxins) in them.[16], [37]–[39] The *cagA* gene has been detected in the genome of *H. pylori* in gastric biopsies of Iranian dogs, particularly those with gastric ulcers.[13] In patients with gastric carcinoma associated with *H. pylori* infection, *cagA* and *babA*$_2$ were the most common genes among the virulence factors.[20]

We sought to evaluate the detected genomes of the *Wolinella* strains found in the current study for the presence of *cagA* and *babA* as crucial virulence genes of *H. pylori*. We also evaluated the presence of these virulence genes in the genome of our Helicobacters. The results presented here show that, despite the genomic identity between *Wolinella* and *H. pylori* noted in the reference articles, *cagA* and *babA* virulence genes were not detected in any of the *Wolinella* strain samples or in the non-pyloric Helicobacters.

5. Conclusions

The current study found that, among members of the Helicobacteraceae family, *Wolinella* spp. was the predominant population over genus *Helicobacter* organisms in the oral cavity of dogs. Additionally, no *Helicobacter pylori* was detected in any of the samples. The incidence of *Helicobacter* infections in the current study was generally associated with non-pylori Helicobacters. These results were corroborated by the results of published reference articles. The results clearly show that, despite the great genomic homology between *W. succinogenes* and *H. pylori* discussed by Baar et al.[18], the *cagA* and *babA* virulence genes were not identified in any of the oral samples taken from dogs in the current study.

6. Patents

Author Contributions: Conceptualization, Bahar Nayeri-Fasaei and Shahram Jamshidi; Data curation, Bahar Nayeri-Fasaei and Shahram Jamshidi; Formal analysis, Bahar Nayeri-Fasaei and Shahram Jamshidi; Funding acquisition, Bahar Nayeri-Fasaei; Investigation, Zahra Jahanshiri; Methodology, Zahra Jahanshiri; Project administration, Bahar Nayeri-Fasaei and Shahram Jamshidi; Software, Bahar Nayeri-Fasaei; Supervision, Taghi Zahraei-Salehi; Validation, Bahar Nayeri-Fasaei and Shahram Jamshidi; Visualization, Zahra Jahanshiri; Writing – original draft, Zahra Jahanshiri; Writing – review & editing, Bahar Nayeri-Fasaei, Shahram Jamshidi and Taghi Zahraei-Salehi.

Funding: This research was funded by Faculty of Veterinary Medicine-University of Tehran, grant number 3941

Institutional Review Board Statement: The animal study protocol was approved by the Institutional Review Board (or Ethics Committee) of Faculty of Veterinary Medicine-University of Tehran (protocol code IR.UT.VETMED.REC.1401.005 and date of approval: 13 February 2022).

Informed Consent Statement: Not applicable.

Data Availability Statement: The accession of sequenced gene presented in this study is openly available in https://www.ncbi.nlm.nih.gov/nuccore/ON845539.1?report=GenBank

Acknowledgments: The authors would like to thank Dr. B. Nayeri-Fasaei at the Department of Microbiology, Faculty of Veterinary Medicine at the University of Tehran in Iran and Dr. S. Jamshidi at the Department of Clinical Sciences, Faculty of Veterinary Medicine at the University of Tehran. This project would not have been possible without their supervision and support. The author also wishes to thank Dr. T. Zahraei-Salehi at the Department of Microbiology, Faculty of Veterinary Medicine at the University of Tehran for his generous support and guidance. The first author is also grateful to Dr. I. Ashrafi-Tamai at the Department of Microbiology, Faculty of Veterinary Medicine at the University of Tehran for his invaluable advice during this project.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. H. Berlamont *et al.*, "Differentiation of gastric helicobacter species using maldi-tof mass spectrometry," *Pathogens*, vol. 10, no. 3, p. 366, Mar. 2021, doi: 10.3390/pathogens10030366.
2. L. P. Andersen, "New Helicobacter species in humans," *Digestive Diseases*, vol. 19, no. 2. Karger Publishers, pp. 112–115, 2001, doi: 10.1159/000050664.
3. C. E. Harvey, "Periodontal disease in dogs. Etiopathogenesis, prevalence, and significance.," *The Veterinary clinics of North America. Small animal practice*, vol. 28, no. 5. Elsevier, pp. 1111–1128, 1998, doi: 10.1016/S0195-5616(98)50105-2.
4. M. P. Riggio, A. Lennon, D. J. Taylor, and D. Bennett, "Molecular identification of bacteria associated with canine periodontal disease," *Vet. Microbiol.*, vol. 150, no. 3–4, pp. 394–400, Jun. 2011, doi: 10.1016/j.vetmic.2011.03.001.
5. F. Haesebrouck *et al.*, "Gastric helicobacters in domestic animals and nonhuman primates and their significance for human health," *Clin. Microbiol. Rev.*, vol. 22, no. 2, pp. 202–223, Apr. 2009, doi: 10.1128/CMR.00041-08.
6. C. Recordati *et al.*, "Detection of Helicobacter spp. DNA in the oral cavity of dogs," *Vet. Microbiol.*, vol. 119, no. 2, pp. 346–351, Jan. 2007, doi: https://doi.org/10.1016/j.vetmic.2006.08.029.
7. D. De Groote *et al.*, "Detection of Non-pylori Helicobacter species in 'Helicobocter heilmannii'-infected humans," *Helicobacter*, vol. 10, no. 5, pp. 398–406, Oct. 2005, doi: 10.1111/j.1523-5378.2005.00347.x.
8. F. Arfaee *et al.*, "PCR-based diagnosis of Helicobacter species in the gastric and oral samples of stray dogs," *Springer*, 2012, doi: 10.1007/s00580-012-1584-5.
9. M. Craven *et al.*, "Evaluation of the Helicobacteraceae in the oral cavity of dogs Melanie," *Am. J. Vet. Res.*, vol. 72, no. 11, pp. 1476–1481, 2011, doi: 10.2460/ajvr.72.11.1476.
10. N. Nowroozilarki, S. Jamshidi, T. Zahraei Salehi, and S. Kolahian, "Identification of Helicobacter and Wolinella spp. in Oral Cavity of Toy Breed Dogs With Periodontal Disease," *Top. Companion Anim. Med.*, vol. 32, no. 3, pp. 96–99, 2017, doi: 10.1053/j.tcam.2017.07.004.

11. A. Shojaee Tabrizi, S. Jamshidi, A. Oghalaei, T. Zahraei Salehi, A. Bayati Eshkaftaki, and M. Mohammadi, "Identification of Helicobacter spp. in oral secretions vs. gastric mucosa of stray cats," *Vet. Microbiol.*, vol. 140, no. 1–2, pp. 142–146, Jan. 2010, doi: 10.1016/j.vetmic.2009.07.019.
12. F. S. Abdi, S. Jamshidi, F. Moosakhani, F. Sasani, and M. Bateman, "Detection of Helicobacter spp. DNA in the colonic biopsies of stray dogs: Molecular and histopathological investigations," *Diagn. Pathol.*, vol. 0, no. 0, pp. 1–6, Mar. 2014, doi: 10.1186/1746-1596-9-50.
13. S. Torkan and M. H. S. Shahreza, "VacA, cagA, iceA and oipA genotype status of Helicobacter pylori isolated from biopsy samples from Iranian dogs," *Trop. J. Pharm. Res.*, vol. 15, no. 2, pp. 377–384, 2016, doi: 10.4314/tjpr.v15i2.22.
14. M. A. García-Amado *et al.*, "Non-pylori helicobacteraceae in the upper digestive tract of asymptomatic Venezuelan subjects: Detection of Helicobacter cetorum-like and candidatus Wolinella africanus-like DNA," *Helicobacter*, vol. 12, no. 5, pp. 553–558, 2007, doi: 10.1111/j.1523-5378.2007.00526.x.
15. U. R. M. Bohr *et al.*, "Detection of a Putative Novel Wolinella Species in Patients with Squamous Cell Carcinoma of the Esophagus," *Helicobacter*, vol. 8, no. 6, pp. 608–612, Dec. 2003, doi: 10.1111/j.1523-5378.2003.00186.x.
16. C. Baar *et al.*, "Complete genome sequence and analysis of Wolinella succinogenes," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 100, no. 20, pp. 11690–11695, 2003, doi: 10.1073/pnas.1932838100.
17. S. Ansari and Y. Yamaoka, "Helicobacter pylori virulence factors exploiting gastric colonization and its pathogenicity," *Toxins*, vol. 11, no. 11. 2019, doi: 10.3390/toxins11110677.
18. M. Sozzi, M. Crosatti, S. K. Kim, J. Romero, and M. J. Blaser, "Heterogeneity of Helicobacter pylori cag genotypes in experimentally infected mice," *FEMS Microbiol. Lett.*, vol. 203, no. 1, pp. 109–114, 2001, doi: 10.1016/S0378-1097(01)00338-X.
19. Y. Yamaoka, M. Kita, T. Kodama, N. Sawai, and J. Imanishi, "Helicobacter pylori cagA gene and expression of cytokine messenger RNA in gastric mucosa," *Gastroenterology*, vol. 110, no. 6, pp. 1744–1752, 1996, doi: 10.1053/gast.1996.v110.pm8964399.
20. S. F. Asl, M. Pourvahedi, A. Mojtahedi, and M. Shenagari, "Analysis of babA, cagE and cagA Genes in Helicobacter pylori from Upper Gastric Patients in the North of Iran," *Infect. Disord. - Drug Targets*, vol. 19, no. 3, pp. 274–278, 2018, doi: 10.2174/1871526518666180515113218.
21. C. E. Greene and J. E. Sykes, *Infectious Diseases of the Dog and Cat*. Elsevier Health Sciences, 2013.
22. S. M. Marretta, "The common and uncommon clinical presentations and treatment of periodontal disease in the dog and cat.," *Seminars in veterinary medicine and surgery (small animal)*, vol. 2, no. 4. pp. 230–240, 1987, Accessed: Dec. 05, 2021. [Online]. Available: https://europepmc.org/article/med/3326087.
23. A. L. Griffen, M. R. Becker, S. R. Lyons, M. L. Moeschberger, and E. J. Leys, "Prevalence of Porphyromonas gingivalis and periodontal health status," *J. Clin. Microbiol.*, vol. 36, no. 11, pp. 3239–3242, 1998, doi: 10.1128/jcm.36.11.3239-3242.1998.
24. B. J. Marshall, J. A. Armstrong, D. B. McGechie, and R. J. Glancy, "Attempt to fulfil Koch's postulates for pyloric campylobacter," *Med. J. Aust.*, vol. 142, no. 8, pp. 436–439, 1985, doi: 10.5694/j.1326-5377.1985.tb113443.x.
25. I. Adler *et al.*, "Helicobacter pylori and oral pathology: Relationship with the gastric infection," *World Journal of Gastroenterology*, vol. 20, no. 29. Baishideng Publishing Group Inc, pp. 9922–9935, 2014, doi: 10.3748/wjg.v20.i29.9922.
26. A. Meining, G. Kroher, and M. Stolte, "Animal reservoirs in the transmission of Helicobacter heilmannii: Results of a questionnaire-based study," *Scand. J. Gastroenterol.*, vol. 33, no. 8, pp. 795–798, 1998, doi: 10.1080/00365529850171422.
27. D. R. Elliott, M. Wilson, C. M. F. Buckley, and D. A. Spratt, "Cultivable oral microbiota of domestic dogs," *J. Clin. Microbiol.*, vol. 43, no. 11, pp. 5470–5476, Nov. 2005, doi: 10.1128/JCM.43.11.5470-5476.2005.
28. M. J. Wolin, E. A. Wolin, and N. J. Jacobs, "CYTOCHROME-PRODUCING ANAEROBIC VIBRIO, VIBRIO SUCCINOGENES, SP. N," Accessed: Dec. 01, 2021. [Online]. Available: https://journals.asm.org/journal/jb.
29. U. R. M. Bohr, A. Primus, A. Zagoura, B. Glasbrenner, T. Wex, and P. Malfertheiner, "A group-specific PCR assay for the detection of Helicobacteraceae in human gut," *Helicobacter*, vol. 7, no. 6, pp. 378–383, 2002, doi: 10.1046/j.1523-

5378.2002.00113.x.

30. M. Contreras, A. Morales, M. A. García-Amado, M. De Vera, V. Bermúdez, and P. Gueneau, "Detection of Helicobacter-like DNA in the gastric mucosa of Thoroughbred horses," *Lett. Appl. Microbiol.*, vol. 45, no. 5, pp. 553–557, Nov. 2007, doi: 10.1111/j.1472-765X.2007.02227.x.
31. A. P. A. Oxley, M. Powell, and D. B. McKay, "Species of the family Helicobacteraceae detected in an Australian sea lion (Neophoca cinerea) with chronic gastritis," *J. Clin. Microbiol.*, vol. 42, no. 8, pp. 3505–3512, Aug. 2004, doi: 10.1128/JCM.42.8.3505-3512.2004.
32. R. Elhelw, M. Elhariri, E. Ragab, M. Kadry, and D. Hamza, "Dog as Potential Source of Helicobacter pylori in Egypt: Public Health Significance," *World's Vet. J.*, vol. 10, no. 3, pp. 446–450, 2020, doi: 10.36380/scil.2020.wvj55.
33. S. Kubota-Aizawa *et al.*, "Transmission of Helicobacter pylori between a human and two dogs: A case report," *Helicobacter*, vol. 26, no. 3, p. e12798, Jun. 2021, doi: 10.1111/hel.12798.
34. K. Ishihara, T. Miura, R. Kimizuka, Y. Ebihara, Y. Mizuno, and K. Okuda, "Oral bacteria inhibit Helicobacter pylori growth," *FEMS Microbiol. Lett.*, vol. 152, no. 2, pp. 355–361, Jul. 2006, doi: 10.1111/j.1574-6968.1997.tb10452.x.
35. K. Okuda, K. Ishihara, T. Miura, A. Katakura, H. Noma, and Y. Ebihara, "Helicobacter pylori may have only a transient presence in the oral cavity and on the surface of oral cancer," *Microbiol. Immunol.*, vol. 44, no. 5, pp. 385–388, 2000, doi: 10.1111/j.1348-0421.2000.tb02510.x.
36. C. Troncoso *et al.*, "MALDI-TOF MS and 16S RNA Identification of Culturable Gastric Microbiota: Variability Associated with the Presence of Helicobacter pylori," *Microorg. 2020, Vol. 8, Page 1763*, vol. 8, no. 11, p. 1763, Nov. 2020, doi: 10.3390/MICROORGANISMS8111763.
37. A. Covacci, J. L. Telford, G. Del Giudice, J. Parsonnet, and R. Rappuoli, "Helicobacter pylori virulence and genetic geography," *Science*, vol. 284, no. 5418. American Association for the Advancement of Science, pp. 1328–1333, May 21, 1999, doi: 10.1126/science.284.5418.1328.
38. D. J. Bacon *et al.*, "Involvement of a plasmid in virulence of Campylobacter jejuni 81-176," *Infect. Immun.*, vol. 68, no. 8, pp. 4384–4390, 2000, doi: 10.1128/IAI.68.8.4384-4390.2000.
39. J. Parkhill *et al.*, "The genome sequence of the food-borne pathogen Campylobacter jejuni reveals hypervariable sequences," *Nature*, vol. 403, no. 6770, pp. 665–668, Feb. 2000, doi: 10.1038/35001088.