Dog as Potential Source of Helicobacter Pylori in Egypt: Public Health Significance

Rehab Elhelw  
Cairo University Faculty of Veterinary Medicine

Mahmoud Elhariri  
Cairo University Faculty of Veterinary Medicine

Eman Ragab  
Cairo University Faculty of Veterinary Medicine

Sara M Nader  
Cairo University Faculty of Veterinary Medicine

Dalia Hamza (✉ daliahamza@cu.edu.eg)  
Cairo University Faculty of Veterinary Medicine  https://orcid.org/0000-0001-7579-5432

Research

Keywords: H pylori, Dog, Human, 16s sequencing

DOI: https://doi.org/10.21203/rs.3.rs-26490/v1

License: ☇ ☀ This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background: Helicobacter species is a group of taxonomically related Gram negative, microaerophilic bacteria, which are known to colonize the gastrointestinal and biliary tracts of human and different animal species. The objective of the present study was to determine the occurrence of H. pylori in owned dog and its role in transmission of H pylori to the dog owners.

Method: Human gastric biopsies (n=80) and Canine stool (n=60) were collected and examined by nested PCR. The PCR positive samples from human and dog isolates were further subjected to partial 16s sequencing.

Result: H pylori was proved in 62.5% and 91.6% of owned dog and dog owners, respectively. The 16s sequencing of the isolates from human and dog were homologous.

Conclusion: This study showed higher occurrence of H pylori in both human and dogs in Egypt. Zoonotic transmission between dogs and human is probable and reflects a public health concern.

Background

_Helicobacter pylori_ is a spiral, Gram-negative microaerophilic bacteria which prefer commonly the acidic medium, because of its ability to produce urease [1]. It was considered to be the second predisposing cause of cancer associated deaths and the fourth cancer causing factor all over the world [2]. It was reported that above half of the world’s people with higher percentage from adults are affected by _Helicobacter pylori_ infection especially in developing countries [3].

_Helicobacter_ species were recorded to inhabit the gastric and intestinal mucosa of humans, pet animals as (dogs, cats), avian species, as well as wild animals as monkeys [4, 5]. In human, _H. pylori_ mainly invade the mucosa of human stomach. The majority may develop to asymptomatic gastritis, nevertheless 10% of infections may progress to cause gastric or duodenal ulcers, and 1% may develop to gastric carcinoma [6]. It is well known that there is a close interaction between human and companion animals which provide a great chance for sharing of many zoonotic diseases. Most of these infections in humans initiate from animals, including dogs, through direct contact [7, 8].

Domestic animals especially dogs were charged to be a common source of infection of _Helicobacter_ to human since it was founded a similarity between helicobacteria isolated from both canine stomach and affected human cases with gastritis [9, 10]. Many studies globally reported that the majority of dogs affected with gastric ulcer may act as a reservoir of _H. pylori_ moreover; it can be the initiator host of that pathogen. These studies were reported in many countries as Belgium [11], Thailand [12], Italy [13], Iran [14], and Egypt [15].The prevalence of gastric _Helicobacter_ infection in dogs was reported to range between 61–100% without known route of transmission [16–19].
In dogs, spiral-shaped bacteria are commonly found in the stomach. They are present in 67 to 86% of clinically healthy dogs and in 61 to 100% of dogs presenting chronic vomiting [13, 20]. *Helicobacter* spp. was also isolated from saliva, dental plaque, and feces of dogs which is considered a good prove for the suggestion of transmission by these animals, oro-oral or oro-fecal [21] and proposed to be reservoir and source of *H. pylori* affection. The same condition in people with *H. pylori* infection because oral–oral and faecal-oral are considered possible routes of transmission [22].

The relationship between pet ownership or frequent exposure to dogs and infection with different gastric *Helicobacter* species was assessed [23]. The evidence of *Helicobacter pylori* transmission from dogs to humans, enhance the need for *Helicobacter* detection and treatment in accompanying animals [24].

Therefore, eradication of *Helicobacter* infections in dogs that have close contact with humans should be considered as one of the methods to control this zoonotic infection. This work was aimed to study the occurrence of *H. pylori* infection in dog and its role in human transmission.

**Method**

**Sample collection**

Human gastric biopsies (n = 80) were collected from different private laboratories with the history of dog owner after signed approval of patients who were informed with the obtained results. Canine stool (n = 60) was collected from hospital of the faculty of veterinary medicine, Cairo university and other private veterinary clinics. Biopsies were collected in sterile tubes containing BHI broth [Merck-Germany] and 5% non-activated fetal calf serum and transferred on ice to the laboratory.

**Molecular identification of *Helicobacter pylori* by nested PCR targeting 16S rRNA gene**

DNA was extracted from stool samples using [QIAamp DNA Stool Mini Kit] according to the manufacturer's instructions. While for gastric biopsies, DNA was extracted by using a modification of a previously described method [25]. Briefly, the thawed biopsy samples were homogenized in Griffith’s tubes containing 400 ml of sterile saline[0.85% [wt/vol]], transferred to 1.5-ml screw-cap Eppendorf tubes, and centrifuged[10,000 3 g for 2 min] and the supernatants were discarded. Pellets were resuspended in extraction buffer [20 mM Tris-HCl [pH 8.0], 0.5% [vol/vol] Tween 20 and proteinase K [0.5 mg/ml], vortexed for 2–5 s, and incubated at 56 °C for 1 h and then 100 °C for 10 min. DNA extracts were stored at −20 °C until required.

A nested PCR assay targeted the 16S rRNA gene of *H. pylori* was performed with primer pairs Hp1, Hp2 and Hp3 (Table 1), [26]. The temperature profile was as follows: 30 s at 95 °C, 30 s at 55or 60 °C, and 30 s at 72 °C. The last cycle was identical, except that the 72 °C extension period was increased to 5 min and the mixture was subsequently refrigerated at 4 °C before analysis. For nested PCR, 25 cycles were used
for each round of amplification. PCR products were analyzed on a 2% agarose electrophoresis gel stained with ethidium bromide.

| Genes                              | Primers     | Sequences (5’ – 3’)              | PCR product size (bp) |
|------------------------------------|-------------|----------------------------------|-----------------------|
| Helicobacter genus-specific-16s rRNA | C97-F       | GCTATGACGGGTATCC                 | 1200                  |
|                                    | C05-R       | ACTTCACCCCAGTCG CTG             |                       |
| Helicobacter *pylori*-specific-16s rRNA | HP1-R       | CTGGAGAGACTAAGC CCTCC           | 109                   |
|                                    | HP2-F       | ATTACTGACGCTGATT GTGC           |                       |
|                                    | HP3-F       | AGGATGAAGGTTAAA GGATT           |                       |

Table 1
Sequences of the primers used in PCR

| Samples   | Total number | Positive H. pylori-specific 16S rRNA gene |
|-----------|--------------|------------------------------------------|
|           | No.      | %       |
| Dog       | 80       | 50        | 62.5        |
| Human     | 60       | 55        | 91.6        |
| Total     | 140      | 105       | 75          |

Table 2
occurrence of *H. pylori*-specific 16S rRNA gene in human and canine samples
Sequencing of *Helicobacter pylori*-specific 16S rRNA PCR positive samples.

In order to study the relation between *H. pylori* from human and dog, the extracted DNA from 16S rRNA PCR positive samples were amplified for Helicobacter genus-specific 16 s using C97 and C05 primers (Table 1) [27]. The temperature profile was as follows 94 °C, 1 min; 55 °C, 2.5 min; 72 °C, 3 min (35 cycles).

The PCR products were purified using Qiaquick purification kit (Qiagen) and were sequenced using Big Dye Terminator V3.1 sequencing kit (Applied Biosystems, Waltham, MA, USA). The obtained nucleotide sequences were compared with those in the Public Database using the NCBI-BLAST server and were deposited in the GeneBank under accession number (MN901212 and MN901172).

Phylogenetic analysis.

The obtained nucleotide sequences were compared with those available in public domains using the NCBI-BLAST server. Sequences were downloaded and imported into the BioEdit program version 7.0.1.4 for multiple alignments using the BioEdit Clustal W program. Phylogenetic analysis was performed with the MEGA program version 7 using the neighbor-joining approach.

Discussion
*H. pylori* is a gram negative, spiral shaped bacterium of the human stomach. It is considered as one of the most contentious bacteria in the world. *H. pylori* is responsible for peptic ulcer, gastritis, lymphoma, duodenal ulcer, and gastric cancer [28,29].

Recorded findings revealed that dogs may play an imperative impact in transmission of *H. Pylori* to human [13, 15]. Gastrointestinal *H. pylori* infections are routine in dogs [10, 19].

Invasive methods for the detection of Helicobacter spp. are still commonly used, involving gastroscopy and collection of a biopsy sample for the performance of rapid urease test and histopathological exam. Currently, the use of the detection of bacterial DNA by PCR has been suggested for the identification of Helicobacter spp. [30].

In the current study, the occurrence of *H pylori* recovered from dog stool was 62.5%. In investigation carried by [31] in China, dogs are the main home-reared animals, and it was found that the positive rate of *H. pylori* infection was higher in populations rearing dogs. Epidemiological data revealed that rearing dogs are a risk factor for *H pylori* infection and it was inferred that dogs may be the potential storage source of bacteria [32]. Obviously a study conducted by [26] showed that the higher rates were obtained from dogs gastric biopsies 76.6% (46/60), 89.1% (41/46) in PCR and cultivation respectively.

The *H. pylori* infection rate is lower in developed countries than in developing countries [33], the rate of *H. pylori* infection is generally lower than 30% in developed countries, while its infection rate might be as high as 50–70% in developing countries. Some studies have pointed out that the poor economic status and a lower degree of culture may induce higher *H. pylori* infection rates [34].

Low levels of hygiene used for maintenance of dogs, their close contact with stray animals could be a risk factor in increasing the prevalence of Helicobacter. Moreover, a research was done by [35] in household dogs raised under hygienic conditions and fed with cooked food revealed a low prevalence (8.66%). Although there is a significant presence of helicobacteria in dogs, it is not possible to relate it with gastric alterations in these animals [36–38].

The virulence of Helicobacter in human is established by [39] who mentioned that this bacterium adheres to the gastric mucosa by an adhesine found on its surface called BabB that facilitate the entrance of antigenic products inside the host. On other hand, there is another mechanisms for its pathogencity by production of cytokines Cag A and VacA [10].

In this study, *H pylori* was proved in 91.6% of dog owners.

The prevalence of helicobacter was investigated in 290 patients from Macau, China [40]. In Egypt, serological detection of *H. pylori* was done by [27] in dog and human revealed that 37.2% (35/94), 44.4% (40/90) respectively was positive. Twenty–nine serum samples which taken from owners of infected dog revealed 51.7% were found to have antibodies to *H. pylori*. This indicates the zoonotic importance and the possibility of transmission of disease between dog and its owner.
It is clear that some animals including cats, dogs, sheep, may be transitory infected by *H. pylori*, but their roles in the route of transmission to humans are not proved [41].

The investigations revealed that dogs, especially those suffering from gastric ulcer, may be the reservoir of *H. pylori* and/or might be the original host of this bacterium [14]. This finding has been documented previously from all-around the world including Egypt where [15] suggested that *Helicobacter* colonizes the stomachs and intestines of humans and several animal species, such as cats, dogs and might have jumped quite recently from animal hosts to people. Because it has been possible to transfer from humans to animals it is reasonable to suppose that animals might have been the original source of the bacterium.

In this study, the phylogenetic relation between human and dog samples were showed 100% homology.

Human infection by *H. pylori* likely post-dated the evolution of humans and resulted from a host jump from a different animal[42]. Host jumps are not necessarily unlikely, because the stomachs of multiple animals are infected by diverse *Helicobacter* species, whose phylogeny is incongruent with that of their hosts. Indeed, the closest known relative of *H. pylori* is *H. acinonychis*, which infects large felines and seems to have arisen by a host jump from humans [43].

**Conclusion**

The occurrence of *H pylori* in the high percentage in dog owners proved the role of the owned dogs in the transmission of this pathogen. The evidence of *Helicobacter pylori* transmission from dogs to humans, enhance the need for *Helicobacter* detection and treatment in accompanying animals.

**Declarations**

**Authors’ contributions**

All authors contributed to the collection of samples, isolation of strains, performing the molecular detection of target genes, analysis and interpretation of the data as well as writing the manuscript. All authors read and approved the final manuscript.

**Author details**

1 Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, PO Box 12211, Giza, Egypt.

2 Department of Zoonoses, Faculty of Veterinary Medicine, Cairo University, PO Box 12211, Giza, Egypt.

**Acknowledgements**
Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**

All data generated or analysed during this study are included in this published article.

**Ethics approval and consent to participate**

The study was conducted according to ethical guidelines approved by Faculty of Veterinary Medicine, Cairo University.

**Funding**

The authors declare that they did not have any funding source or grant to support their research work.

**References**

1. Siqueira JS, Lima PS, Barreto AS, Quintans.Júnior LJ. Aspectos Gerais nas Infecções por Helicobacter pylori Revisão. RBAC. 2007;39 (1):9.13.
2. The Globocan Project,. International Agency for Research on Cancer, The Globocan Project (online) (access in 2010). http://globocan.iarc.fr/.
3. Frenck Jr RW, Clemens J. Helicobacter in the developing world. Microbes and infection. 2003;5(8):705.13.
4. Abdi FS, Jamshidi S, Moosakhani F, Sasani F. Detection of Helicobacter spp. DNA in the colonic biopsies of stray dogs: molecular and histopathological investigations. Diagnostic pathology. 2014 ;9(1):50.
5. Hong S, Chung Y, Kang WG, Choi YS, Kim O. Comparison of three diagnostic assays for the identification of Helicobacter spp. in laboratory dogs. Lab Anim Res. 2015 ;31(2):86.92.
6. Beswick EJ, Suarez G, Reyes VE. Helicobacter pylori and host interactions that influence pathogenesis. World J Gastroenterol 2006; 12:5599–605.
7. Meining A, Kroher G, Stolte M. Animal reservoirs in the transmission of Helicobacter heilmannii: results of a questionnaire-based study. Scandinavian journal of gastroenterology. 1998;33(8):795.8.
8. Haesebrouck F, Pasmans F, Flahou B, Chiers K, Baele M, Meyns T, et al. Gastric helicobacters in domestic animals and nonhuman primates and their significance for human health. Clinical microbiology reviews. 2009;22(2):202.23.
9. Soghra Abdi F, Jamshidi S, Moosakhani F, Sasani F. Detection of Helicobacter spp. DNA in the colonic biopsies of stray dogs: molecular and histopathological investigations. Diagn Pathol 2014; 9: 50.
10. Okubo BM, Ricci.Azevedo R, Zobiole NN, Buccini DF, Moreno SE. PREVALENCE OF Helicobacter spp. IN DOGS FROM CAMPO GRANDE.MS. Ciência Animal Brasileira. 2017;18.
11. Van den Bulck K, Decostere A, Baele M, Driessen A, Debongnie JC, Burette A, Stolte M, Ducatel1le R, Haesebrouck F. Identification of non.Helicobacter pylori spiral organisms in gastric samples from humans, dogs, and cats. J Clin Microbiol 2005; 43: 2256.2260.
12. Pirarat N, Makbunsri T, Sukkamon S, Amornchailertrat S, Rungsipipat A, Su- nyasootcharee B. The relationship between pathological gastric changes and Helicobacter spp. in dog. Thai J Vet Med 2003; 33: 73.80
13. Recordati C, Gualdi V, Tosi S, Facchini RV, Pengo G, Luini M, et al. Detection of Helicobacter spp. DNA in the oral cavity of dogs. Veterinary microbiology. 2007 31;119(2.4):346.51.
14. Torkan S, Shahreza MH. VacA, CagA, IceA and OipA genotype status of Helicobacter pylori isolated from biopsy samples from Iranian dogs. Tropical Journal of Pharmaceutical Research. 2016;15(2):377.84.
15. Abdel.Raouf M, Abdel.Gleel Y, Enab A. Study on the role of pet animals for Helicobacter pylori transmission. J Am Sci 2014;10:20.8.
16. Eaton KA, Dewhirst FE, Paster BJ, Tzellas N, Coleman BE, Paola J, et al. Prevalence and varieties of Helicobacter species in dogs from random sources and pet dogs: animal and public health implications. Journal of clinical microbiology. 1996;34(12):3165.70.
17. Happonen I, Linden J, Saari S, Karjalainen M, Hänninen ML, Jalava K, et al. Detection and effects of helicobacters in healthy dogs and dogs with signs of gastritis. Journal of the American Veterinary Medical Association. 1998;213(12):1767.74.
18. Yamasaki K, Suematsu H, Takahashi T. Comparison of gastric lesions in dogs and cats with and without gastric spiral organisms. Journal of the American Veterinary Medical Association. 1998;212(4):529.33.
19. Wiinberg B, Spohr A, Dietz HH, Egelund T, Greiter-Wilke A, McDonough SP, et al. Quantitative analysis of inflammatory and immune responses in dogs with gastritis and their relationship to Helicobacter spp. infection. Journal of veterinary internal medicine. 2005;19(1):4.14.
20. Hwang CY, Han HR, Youn HY. Prevalence and clinical characterization of gastric Helicobacter species infection of dogs and cats in Korea. Journal of Veterinary Science. 2002;3(2):123.34.
21. Souza ML, Kobayasi S, Rodrigues MA, Saad.Hossne R, Naress LE. Prevalência de Helicobacter em câes oriundos do biotério central da Universidade Estadual de São Paulo (UNESP).Botucatu. Acta Cirurgica Brasileira. 2004;19(5):565.70.
22. Brown LM. Helicobacter pylori: epidemiology and routes of transmission. Epidemiologic reviews. 2000;22(2):283.97.
23. Chung TH, Kim HD, Lee YS, Hwang CY. Determination of the prevalence of Helicobacter helimannii.like organisms type 2(HHLO.2) infection in humans and dogs using non.invasive genus/species.specific PCR in Korea. J Vet Med Sci 2014; 76:73–9.
24. Nowroozilarki N, Jamshidi S, Salehi TZ, Kolahian S. Identification of Helicobacter and Wolinella spp.
In oral cavity of toy breed dogs with periodontal disease. Topics in companion animal medicine. 2017;32(3):96.9. http://dx.doi.org/10.1053/j.tcam.2017.07.004.

25. A. Marais L, Monteiro A, Occhialini, et al., Direct detection of Helicobacter Pylori resistance to macrolides by a polymerase chain reaction/DNA enzyme immunoassay in gastric biopsy specimens. Gut. 1999; 44: 463–467.26. Hamza D, Elhelw R, Elhariri M, Ragab E. Genotyping and antimicrobial resistance patterns of Helicobacter pylori in human and dogs associated with A2142G and A2143G point mutations in clarithromycin resistance. Microbial pathogenesis. 2018 ;123:330.8.

27. Elhariri M, Elhelw R, Hamza D, El.Mahallawy HS . Serologic evidence and risk factors for Helicobacter pylori infection in animals and humans. J Infect Dev Ctries .2017;11:414.419. doi: 10.3855/jidc.9339.

28. Atapoor S, Dehkordi FS, Rahimi E. Detection of Helicobacter pylori in various types of vegetables and salads. Jundishapur J Microbiol 2014;7: e10013.

29. Ghorbani F, Gheisari E, Dehkordi FS. Genotyping of vacA alleles of Helicobacter pylori strains recovered from some Iranian food items. Trop J Pharm Res 2016;15:1631.6.

30. Jankowski R, Nguyen DT, Poussel M, Chenuel B, Gallet P, Rumeau C. Sinusologie. Annales françaises d’Oto.rhino.laryngologie et de Pathologie Cervico.faciale. 2016;133(4):237.43.

31. Zou J, Xiao YQ, Cheng YF, Ren XY, Li SW, Gang D. Investigation of Helicobacter pylori Infection and Its Related Factors in the Tianjin Binhai Area, China. Jundishapur Journal of Microbiology. 2019;12(9):1.0.

32. Dore MP, Malaty HM, Graham DY, Fanciulli G, Delitala G, Realdi G. Risk factors associated with Helicobacter pylori infection among children in a defined Geographic Area. Clin Infect Dis. 2002;35(3):240–5. doi: 10.1086/341415.

33. Pounder RE, Ng D. The prevalence of Helicobacter pylori infection in different countries. Alimentary pharmacology & therapeutics. 1995; 9:33.9.

34. Hu FL. Helicobacter pylori infection is a research topic involving multiple disciplines. Zhonghua Yi Xue Za Zhi.2008;88(22):1513–5.

35. Bolandi A, Torkan S, Alavi I. Genotyping pattern of the vacuolating cytotoxin A and cytotoxin associated gene A of the Helicobacter pylori strains detected in fecal samples of household dogs. Microbiology Research. 2017;8(2).

36. Rossi G, Rossi M, Vitali CG, Fortuna D, Burrioni D, Pancotto L, Capecchi S, Sozzi S, Renzoni G, Braca G, Del Giudice G. A conventional beagle dog model for acute and chronic infection with Helicobacter pylori. Infection and immunity. 1999;67(6):3112.20.

37. Moutinho FQ, Thomassian A, Watanabe MJ, Suzano SM, Sequeira JL. Prevalência de helicobactérias e alterações na mucosa gástrica de cães saudáveis. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 2007;59(4):1080.3.

38. Takemura LS, Amude AM, Camargo PL, Bracarense AP. Detecção e efeitos de Helicobacter spp. em cães sadios e com sinais de gastrite. Acta Scientiae Veterinariae. 2007;35(s2).

39. Ladeira MSP, Salvadori D M F, Rodrigues MAM. Biopathology of Helicobacter pylori. J. Bras. Patol. Med. Lab. 2003;39 (4 )335.342.

40. Pinto.Ribeiro I, Ferreira RM, Batalha S, Hlaing T, Wong SI, Carneiro F, Figueiredo C. Helicobacter pylori vacA genotypes in chronic gastritis and gastric carcinoma patients from Macau, China. Toxins. 2016;8(5):142.
41. Irena M, Olga G, Ami P. Zoonotic potential of Helicobacter spp. Journal of Microbiology, Immunology and Infection. 2017; 50: 265e269.

42. Dewhirst FE, Shen Z, Scimeca MS, Stokes LN, Boumenna T, et al. Discordant 16S and 23S rRNA gene phylogenies for the genus Helicobacter: Implications for phylogenetic inference and systematics. J Bacteriol. 2005; 187: 6106–6118.

43. Eppinger M, Baar C, Linz B, Raddatz G, Lanz C, et al. Who ate whom? Adaptive Helicobacter genomic changes that accompanied a host jump from early humans to large felines. PLoS Genet. 2006; 2: e120.