



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

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ABSTRACT

Helicobacter species are a group of Gram-negative, microaerophilic bacteria, which are known to colonize the gastrointestinal and biliary tracts of humans and various animal species. The objective of the present study was to determine the prevalence of *Helicobacter pylori* in owned dogs and their role in the transmission of *H. pylori* to the dog owners. For this purpose, 60 gastric biopsy samples from dog owners and 80 stool samples from owned dogs were collected and examined for the presence of *H. pylori* 16s rRNA gene by nested PCR. The PCR positive samples from human and dog isolates were further subjected to partial *Helicobacter* genus-specific 16s rRNA gene sequencing. Phylogenetic analysis based on partial sequence of this gene was performed to determine the relationship between human and dog isolates. *H. pylori* was detected in 62.5% and 91.6% of dog and human samples, respectively. The nucleotide sequence of *Helicobacter* genus-specific 16s rRNA gene of human and dog isolates were similar. In conclusion, this study indicated a high prevalence of *H. pylori* in both dogs and dog owners in Egypt. Zoonotic transmission of *H. pylori* between dogs and humans is probable and represents a public health concern.

Keywords: Dogs, *Helicobacter pylori*, Humans, Phylogenetic analysis, 16s rRNA sequencing

INTRODUCTION

Helicobacter pylori is a spiral, Gram-negative microaerophilic bacterium that prefers commonly the acidic medium, because of its ability to produce urease (Siqueira et al., 2007). It is considered to be the second predisposing cause of cancer-associated deaths and the fourth cancer-causing factor all over the world (The Globocan Project, 2010). It is reported that above half of the world's people with a higher percentage of adults are affected by *H. pylori* infection, especially in developing countries (Frenck and Clemens, 2003).

Helicobacter species inhabits the gastric and intestinal mucosa of humans, pet animals including dogs and cats, avian species, as well as wild animals such as monkeys (Abdi et al., 2014; Hong et al., 2015). In humans, *H. pylori* mainly invade the mucosa of the stomach. The majority of *Helicobacter* infections may develop to asymptomatic gastritis, nevertheless, 10% of infections may progress to cause gastric or duodenal ulcers, and 1% may develop into gastric carcinoma (Beswick et al., 2006). It is well known that there is close contact between humans and companion animals, which poses a great risk of transmitting many zoonotic diseases. Most of these infections in humans initiate from animals, including dogs, through direct contact (Meining et al., 1998; Haesebrouck et al., 2009).

In dogs, spiral-shaped bacteria are commonly found in the stomach. They are present in 67-86% of clinically healthy dogs and 61-100% of dogs presenting chronic vomiting (Hwang et al., 2002; Recordati et al., 2007). Many studies have reported that the majority of dogs affected with gastric ulcers may act as a reservoir of *H. pylori*. These studies have been reported in many countries such as Belgium (Van den Bulck et al., 2005), Thailand (Pirarat et al., 2003), Italy (Recordati et al., 2007), Iran (Torkan and Shahreza, 2016), and Egypt (Abdel-Raouf et al., 2014). The prevalence of gastric *Helicobacter* infection in dogs has been reported to be between 61 to 100% (Eaton et al., 1996; Happonen et al., 1998; Yamasaki et al., 1998; Wiinberg et al., 2005). Also, a relationship has been found between pet ownership or frequent exposure to dogs and infection with different gastric *Helicobacter* species (Chung et al., 2013).

Therefore, domestic animals, especially dogs, are charged to be a common source of *Helicobacter* (Abdi et al., 2014; Okubo et al., 2017). The isolation of *Helicobacter* spp. from saliva, dental plaque, and feces of dogs reinforces the hypothesis of transmission from these animals through oral-oral or fecal-oral routes (Souza et al., 2004). The same condition is observed in humans with *H. pylori* infection, i.e., oral-oral and fecal-oral are considered possible routes of transmission (Brown, 2000). The occurrence of *H. pylori* in dogs and humans enhances the need for *Helicobacter* detection and companion animal treatment (Nowroozilarki et al., 2017). Therefore, eradication of *Helicobacter*

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infections in dogs that have close contact with humans should be considered as one of the methods to control this zoonotic infection.

The aim of this research was to study the prevalence of *H. pylori* infection in owned dogs and dog owners of Egypt and its public health significance.

MATERIALS AND METHODS

Ethical approval

This study was conducted according to ethical guidelines approved by the Faculty of Veterinary Medicine, Cairo University, Egypt. The signed consent for the use of samples was obtained from each patient who participated in the study.

Sample collection

Gastric biopsy samples (n=60) of patients with the history of dog ownership were collected from different hospitals in Giza, Egypt. Canine stool samples (n=80) were collected from the hospital of the Faculty of Veterinary Medicine, Cairo University, and other private veterinary clinics in the Giza governorate. Biopsy and stool samples were collected in sterile tubes containing Brain Heart Infusion (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, (Germany) according to the manufacturer's instructions. While for gastric biopsies, DNA was extracted by a modification of the method described by (Marais et al., 1999). The extracted DNA was stored at -20 °C until required. The nested PCR assay targeting the *16S rRNA* gene of *H. pylori* was performed using primer pairs Hp1, Hp2, and Hp3 (Table 1) (Hamza et al., 2018). The temperature profile was as follows: 30 s at 95 °C, 30 s at 55 or 60 °C, and 30 s at 72 °C. For nested PCR, 25 cycles were used for each round of amplification. PCR products were analyzed on 2% agarose gel electrophoresis stained with ethidium bromide.

Sequencing of *Helicobacter* genus-specific *16S rRNA* gene

To study the relationship between *H. pylori* isolated from human and dog, the extracted DNA from PCR positive samples were amplified for *Helicobacter* genus-specific *16s rRNA* gene using C97 and C05 primers (Table 1) (Elhariri et al., 2017). The temperature profile was as follows: 94 °C for 1 min; 55 °C for 2.5 min; 72 °C for 3 min (35 cycles). The PCR products were purified using a Qiaquick purification kit (Qiagen, Germany) and sequenced using Big Dye Terminator V3.1 sequencing kit (Applied Biosystems, Waltham, MA, USA). The obtained nucleotide sequences from human and dog isolates were submitted to the GenBank under accession numbers MN901212 and MN901172, respectively.

Phylogenetic analysis

The nucleotide sequences in the current study were compared with those available in GenBank databases 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.

Table 1. Primers used in this study

Target genes	Primers	Sequences (5'-3')	PCR product size (base pair)
<i>Helicobacter</i> genus-specific <i>16s rRNA</i>	C97-F	GCT ATG ACG GGT ATC C	1200
	C05-R	ACT TCA CCC CAG TCG CTG	
<i>Helicobacter pylori</i> -specific- <i>16s rRNA</i>	HP1-R	CTGGAGAGACTAAGCCCTCC	109
	HP2-F	ATTACTGACGCTGATTGTGC	
	HP3-F	AGGATGAAGGTTTAAGGATT	

F: forward, R: reverse

RESULTS

H.pylori was detected by nested PCR assay targeting the *16S rRNA* gene of *H. pylori* in 62.5% (50/80) and 91.6% (55/60) of owned dogs and humans, respectively. The nucleotide sequences of *Helicobacter* genus-specific *16s rRNA* from human and dog isolates were located in the same cluster with a bootstrapping value 99% (Figure 1), indicating that they are highly related to each other. The similarity was found between *H. pylori* isolated from both dogs and human cases.

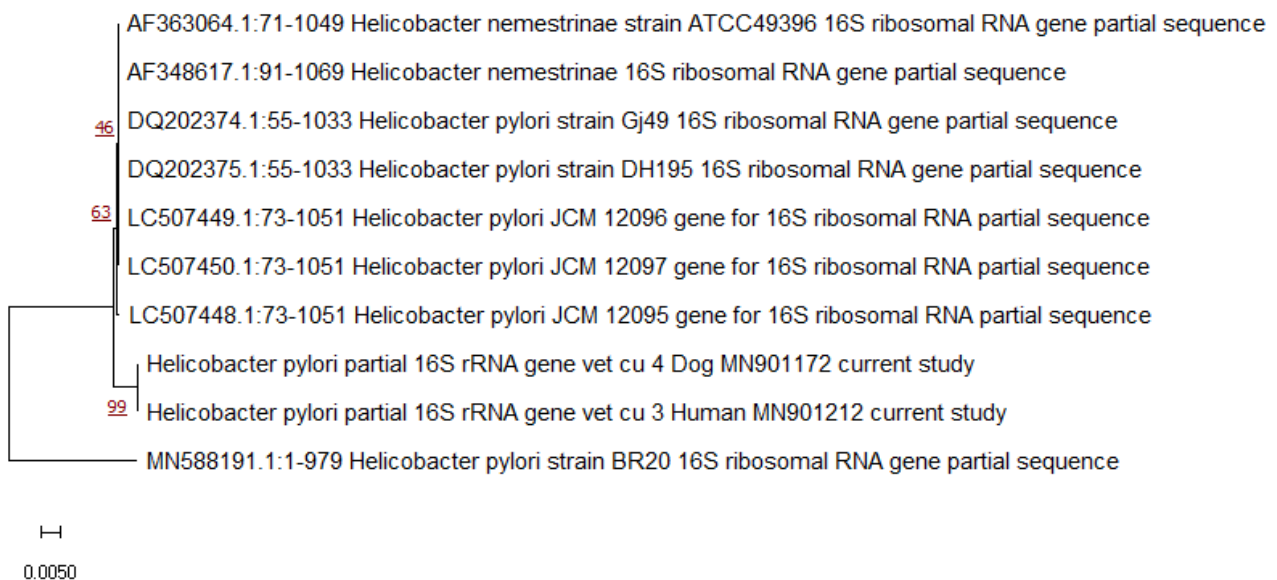


Figure 1. Neighbor-joining tree based on the nucleotide sequences of the partial coding regions of *16s rRNA* gene of *H. pylori* from human and dog isolates. The Evolutionary analysis was performed with MEGA version 7.

DISCUSSION

Previous findings revealed that dogs may play an important role in the transmission of *H. pylori* to humans (Recordati et al., 2007; Abdel-Raouf et al., 2014). Many researches indicated that the prevalence of *H. pylori* is high in dogs and may reach 100% (Wiinberg et al., 2005; Okubo et al., 2017). In the current study, 62.5% of dog stool samples were positive for *H. pylori* by PCR, which is similar to the findings of Hong et al. (2015) who detected *Helicobacter* spp. DNA in feces of laboratory dogs. In a study carried out by Zou et al. (2019) in China, it was found that dogs were the main home-reared animals and the positive rate of *H. pylori* infection was higher in people who breed dogs. Epidemiological data revealed that ownership of dogs is a risk factor for *H. pylori* infection in children in the rural areas and it was concluded that dogs could be a potential source of bacteria (Dore et al., 2002).

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 (Torkan and Shahreza, 2016). A study conducted by Hamza et al. (2018) showed that higher rates of *H. pylori* detection in gastric biopsies of dogs using PCR (76.6%) and cultivation (89.1%).

The prevalence rate of *H. pylori* infection was found to be lower in developed countries than in developing countries. The rate of *H. pylori* infection is generally lower than 30% in developed countries, while it may be as high as 50-70% in developing countries (Pounder and Ng, 1995). Some studies have pointed out that the poor economic status and a lower degree of culture may induce higher *H. pylori* infection rates (Hu, 2008). Poor hygiene conditions and close contact with stray animals could be risk factors that increase the prevalence of *Helicobacter* infections (Bolandi et al. 2017). Moreover, the research conducted by Bolandi et al. (2017) on household dogs raised under hygienic conditions and fed with cooked food revealed a low prevalence (8.66%) of *H. pylori* infection. Although there is a significant presence of *Helicobacter* in dogs, it is not possible to relate it with gastric alterations in these animals (Rossi et al., 1999; Moutinho et al., 2007; Takemura et al., 2007). *H. pylori* infect about 50 % of the world's human population associated with gastric and extra gastric diseases (Bravo et al., 2018). *H. pylori* is responsible for peptic ulcer, gastritis, lymphoma, duodenal ulcer, and gastric cancer (Atapoor et al., 2014; Ghorbani et al., 2016).

In this study, *H. pylori* was detected in 91.6% of dog owners. This differs from El-Shenawy et al. (2017) who reported that the prevalence rate of *H. pylori* in Egyptian patients was 53.1%, but similar to the finding of Abu-Zekry et al. (2013) that *H. pylori* infection was detected by culture method in 70% of gastric biopsy specimens. This difference may be due to a variety of contributing factors, including socioeconomic status, living conditions, and location of each population even in the same country.

In Egypt, serological detection of *H. pylori* was done by Elhariri et al. (2017) in dogs and humans and found that 37.2% and 44.4% were positive, respectively. This indicates the zoonotic importance and the possibility of transmission of disease between dogs and their owners. It is clear that some animals, including cats, dogs, and sheep may be infected by *H. pylori*, but their roles in the transmission to humans are not proved (Mladenova-Hristova et al., 2017).

H. pylori infection in Human probably resulted from a host jump from a different animal (Dewhirst et al., 2005). Host jump is not impossible, in light of the fact that the stomachs of different animals are contaminated with various *Helicobacter* species, whose phylogeny is incongruent with that of their hosts. In fact, the closest known relative of *H. pylori* is *H. acinonychis*, which invade large cats and appears to have emerged by a host jump from humans (Eppinger et al., 2006).

The theory of host jump of *H. pylori* was reinforced by making the phylogenetic relationship between human and dog samples which showing 100% homology between each other and this reflects the possibility of the transmission of this bacteria between dogs and their owners. This finding has been documented previously from all around the world, including Egypt, where (Abdel-Raouf et al., 2014) 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.

CONCLUSION

The high prevalence of *H. pylori* in dog owners proved the role of the owned dogs in the transmission of this pathogen. The evidence of *H. pylori* transmission from dogs to humans enhances the need for *Helicobacter* detection and treatment in pet animals.

DECLARATION

Authors' contributions

Rehab Elhelw, Mahmoud Elhariri, Eman Ragab, Mona Kadry, and Dalia Hamza 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.

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Competing interests

The authors declare that they have no competing interests.

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