

Short Paper

Identification of *Helicobacter* spp. in gastrointestinal tract, pancreas and hepatobiliary system of stray cats

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Summary

The aim of the present study was to determine the presence of *Helicobacter* species in different parts of gastrointestinal tract, hepatobiliary system and pancreas of stray cats. Six different sites at the level of genus, gastric (*H. heilmannii* and *H. felis*) and enterohepatic species of *Helicobacter* were investigated in six cats using species-specific primers by polymerase chain reaction (PCR). Interestingly, DNA of enterohepatic spp. was detected in 1/6 duodenum, 2/6 colon and 1/6 pancreas specimens. Results of sequencing revealed that all of these four positive samples belong to *Helicobacter canis*. While cats have not been considered as a potential zoonotic danger for non-*pylori* *Helicobacter* infections, the results of current study show prompt re-evaluation of that view. To the best of our knowledge, this is the first study about distribution of *Helicobacter* spp. in gastrointestinal tract of cats.

Key words: Cat, Gastrointestinal tract, *Helicobacter*, PCR

Introduction

Helicobacter pylori is a well-known factor in pathogenesis of gastritis, gastric ulcer and gastric cancer in human which is distributed in more than 50% of human population (Salih, 2009). In cats, stomach is usually infected with some large non-*pylori* *Helicobacters* called gastric *Helicobacter*-like organisms or GHLOs (Kenneth, 2011) with infection rates of 78-85% and 13-66.6% for *H. heilmannii* and *H. felis*, respectively (Jalava and Stephan, 1998; Neiger and Simpson, 2000; Akhtardanesh *et al.*, 2006). Studies show that *Helicobacter* spp. not only can be found in stomach but also in oral cavity of cats which may play a role in oral-oral transmission (Ghil *et al.*, 2009; Shojaee Tabrizi *et al.*, 2010).

Enterohepatic *Helicobacter* species like *H. canis*, *H. bilis* and *H. cinaedi* have recently been isolated from oral cavity, stomach, intestine, liver and pancreas of some human patients (Dewhirst *et al.*, 2005) most of which, interestingly, had close contact with dogs and cats, supporting the hypothesis of zoonotic transmission (Swennes *et al.*, 2014). Reviews show that these species were detected in 52% of 27 pet dogs, most of them suffering from inflammatory bowel disease (IBD), in a dog with hepatitis, a colony of Bengal cats with endemic diarrhea and a group of healthy cats (Foley *et al.*, 1999; Shen *et al.*, 2001; Castiglioni *et al.*, 2012; Swennes *et al.*, 2014). According to these scattered data, more comprehensive studies are needed to provide better

understanding of infection both in animals and human. The spatial distribution of *Helicobacter* spp. in dogs has been illustrated in one study (Recordati *et al.*, 2009) but, to the best of our knowledge, it has not been determined in cats yet. So, this study was conducted to determine the presence of *Helicobacter* species in different parts of GI tract, pancreas and hepatobiliary system of stray cats.

Materials and Methods

Animals and sampling procedure

This study has been approved by the Iranian laboratory animal ethics framework under the supervision of the Iranian Society for the Prevention of Cruelty to Animals and Shiraz University Research Council. Six stray cats (mean age = 3 years) which were euthanized in Small Animal Hospital of Shiraz University due to automobile accident during 2010-2013 were included in the study. Samples were taken from three different sites of stomach, duodenum, jejunum, colon, pancreas and gall bladder of each individual under aseptic condition. Each of these triplet samples was immediately transferred into tubes containing 1 ml of sterile phosphate buffered saline (PBS) and stored at -20°C until further analysis.

DNA extraction and PCR assays

DNA was extracted from the specimens using DNeasy tissue kit (Qiagen, Germany) according to the manufacturer's instructions. PCR amplifications were

performed in a final volume of 25 μ L containing 3 μ L of extracted DNA, 2.5 μ L of 10 X PCR buffer (CinnaGen, Iran), 1 mM of dNTPS, 1.5 mM of MgCl₂, 20 pmol of each primer and 0.5 U of TaqDNA polymerase (CinnaGen, Iran). Primer sequences and PCR conditions are presented in Table 1. The resulting PCR products underwent gel electrophoresis [1.5% (w/v) agarose gel with 0.3% ethidium bromide in 10% Tris-borate EDTA buffer (TBE)] and were visualized under UV transilluminator.

Results

Genus-specific PCR identified 6/6 (100%) of the subjects as *Helicobacter* spp.-positive in at least two sites of sampling. Cats No. #3 and #5 had *Helicobacter* spp. DNA in all six locations. Stomach, duodenum, jejunum, colon, pancreas and gall bladder were positive in 5/6, 5/6, 4/6, 5/6, 5/6, 3/6 of specimens, respectively. *Helicobacter heilmannii* was just detected in 3/5 of positive gastric samples. Interestingly, the remaining samples were colonized with neither *H. heilmannii* nor *H. felis*. Results also showed that in *Helicobacter* spp.-positive samples, 1/5 of duodenum, 2/5 of colon and 1/5 of pancreas were enterohepatic-positive (Table 2). Consequently, sequencing revealed that all three samples appear to be *H. canis*.

Discussion

The pathogenic role and exact routes of transmission for non-*pylori Helicobacter* spp. both in human and animals are still unclear and remain to be identified. Several researchers suggest that *Helicobacter* spp. can be acquired by close contact with domestic animals but

further investigations seem to be essential (Meining *et al.*, 1998; Shojaee Tabrizi *et al.*, 2010; Swennes *et al.*, 2014). Considering the fact that cats are popular pets and most of the house-hold cats in Iran originate from the stray population, our study was conducted on this group of animals. To the best of our knowledge, the distribution of *Helicobacter* spp. in gastrointestinal tract of cats, as a potential source of infection and transmission to human and other species, has not been determined.

Previous studies have revealed that gastric *Helicobacter*-like organisms which are routinely found in animals stomach can cause severe gastritis in human (Qualia *et al.*, 2007; Roehrl *et al.*, 2012) and enterohepatic *Helicobacters* can lead to gastritis, cutaneous lesions, cellulitis, and fever with unknown origin, especially in immunocompromised individuals (Burnens *et al.*, 1993; Swennes *et al.*, 2014). Subsequently, some case reports describing isolation of *Helicobacter canis* from human with Crohn's disease and autoimmune hepatitis which interestingly, most had had contact with animals, demonstrates the importance of this group of pathogens (Swennes *et al.*, 2014).

Results of current study show that enterohepatic *Helicobacter* spp. can be found not only in small and large intestine but also in pancreas of cats. Presence of *Helicobacter* spp. in the lumen of GI tract could be a confirmation that faeces of cats are a possible source of zoonotic transmission. However, it should be noted that PCR technique was used and differentiating between live and dead bacteria was impossible in this study.

Moreover, since pancreatic disorders are fairly common in cats, this microorganism may have some pathogenic role in this regard. According to the fact that the cats that were included in this study were stray cats and we do not have any information about their history

Table 1: Oligonucleotide primers and PCR conditions

Target genes	Reference	Primer sequence (5'→3')	PCR fragment (bp)	PCR conditions
16S rRNA (<i>Helicobacter</i> spp.)	Germani <i>et al.</i> (1997)	(F) : AAG GAT GAA GCT TCT AGC TTG CTA (R) : GTG CTT ATT CGT GAG ATA CCG TCA T	398	94°C for 4 min (94°C, 54°C and 72°C) 34 cycles for 40 s 72°C for 4 min
<i>Ure A</i> and <i>ure B</i> (<i>H. felis</i>)	Germani <i>et al.</i> (1997)	(F) : GTG AAG CGA CTA AAG ATA AAC AAT (R) : GCA CCA AAT CTA ATT CAT AAG AGC	241	94°C for 4 min (94°C, 62°C and 72°C) 36 cycles for 50 s 72°C for 4 min
<i>ure B</i> (<i>H. heilmannii</i>)	Neiger <i>et al.</i> (1998)	(F) : GGG CGA TAA AGT GCG CTT G (R) : CTG GTC AAT GAG AGC AGG	580	94°C for 4 min (94°C, 60°C and 72°C) 34 cycles for 50 s 72°C for 4 min
Enteric	Recordati <i>et al.</i> (2009)	(F) : TGA-ATG-CTA-GTT-GTT-GCC-CTG-CCT-G (R) : TCT-CCT-TAG-AGT-GCT-CAG-CCG-AAC-T	345	94°C for 4 min (94°C, 56.5°C and 72°C) 30 cycles for 1 min 72°C for 7 min

Table 2: PCR results assessing gastric and enterohepatic *Helicobacter* spp. in GI tract of six stray cats

Cats No.	Gastric/enterohepatic spp.					
	Stomach	Duodenum	Jejunum	Colon	Pancreas	Gallbladder
1	-/-	+/-	+/-	+/-	c/+	-/-
2	h/-	+/-	+/-	+/-	+/-	-/-
3	+/-	+/c	+/-	+/c	+/-	+/-
4	h/-	+/-	-/-	+/-	-/-	+/-
5	+/-	+/-	+/-	+/c	+/-	+/-
6	h/-	-/-	-/-	-/-	+/-	-/-

- indicates negative result, + indicates 16S rRNA-positive, h indicates *H. heilmannii*, and c indicates *H. canis*

and probable clinical signs, further studies to find the association between the infection and alimentary disorders are highly recommended by investigating the infection in house-hold cats with a clearer history.

In this study, no *Helicobacter felis* was found in specimens. The same results were obtained in some of the previous researches which were done on larger groups of cats (Neiger *et al.*, 1998; Ghil *et al.*, 2009).

In conclusion, according to the literature, it seems that attention is mostly directed to the *Helicobacter pylori* infections in humans and the risk of zoonotic transmission of other *Helicobacter* spp. has been neglected. However, given the results of current study, growing population of stray cats, especially in developing countries like Iran, and the likelihood that some of these cats are adopted by families, careful study about this zoonotic potential is reasonable.

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Conflict of interest

The authors have declared no conflicts of interest.

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