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 µL containing 3 µL of extracted DNA, 2.5 µL 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 (Quaila 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

<table>
<thead>
<tr>
<th>Target genes</th>
<th>Reference</th>
<th>Primer sequence (5´→3´)</th>
<th>PCR fragment (bp)</th>
<th>PCR conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA</td>
<td>Germani et al. (1997)</td>
<td>(F): AAC GAT GAA GCT GCT GAT ACG GTC GTA TA (R): GTC CTT ATT CTT GAG AYA CCG TCA T</td>
<td>398</td>
<td>94°C for 4 min (94°C, 54°C and 72°C) 34 cycles for 40 s 72°C for 4 min</td>
</tr>
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<tr>
<td></td>
<td>Germani et al. (1997)</td>
<td>(F): GTC AAG CAA CTA AAG AYA AAC AAT (R): GCA CCA AAT CTT GAT CAT AAG AGC</td>
<td>241</td>
<td>94°C for 4 min (94°C, 62°C and 72°C) 36 cycles for 50 s 72°C for 4 min</td>
</tr>
<tr>
<td></td>
<td>Neiger et al. (1998)</td>
<td>(F): GGS CCA TAA AGG GGC GCT G (R): CTA GTC AAT GAG AAC AGG</td>
<td>580</td>
<td>94°C for 4 min (94°C, 60°C and 72°C) 54 cycles for 50 s 72°C for 4 min</td>
</tr>
<tr>
<td></td>
<td>Recordati et al. (2009)</td>
<td>(F): TCA-ATG-CTT-GCT-GT-CC-CGC-GTT-TG (R): TCT-CGT-TAG-ACT-GAT-CAG-GCG-TG-TAC</td>
<td>345</td>
<td>94°C for 4 min (94°C, 56.5°C and 72°C) 30 cycles for 1 min 72°C for 7 min</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Cats No.</th>
<th>Stomach</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Colon</th>
<th>Pancreas</th>
<th>Gallbladder</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>c+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>2</td>
<td>h/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>3</td>
<td>+/-</td>
<td>+/c</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>4</td>
<td>h/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>5</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>6</td>
<td>h/-</td>
<td>+/-</td>
<td>+/-</td>
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<td>+/-</td>
</tr>
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</table>

- 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.

Acknowledgement

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Conflict of interest

The authors have declared no conflicts of interest.

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